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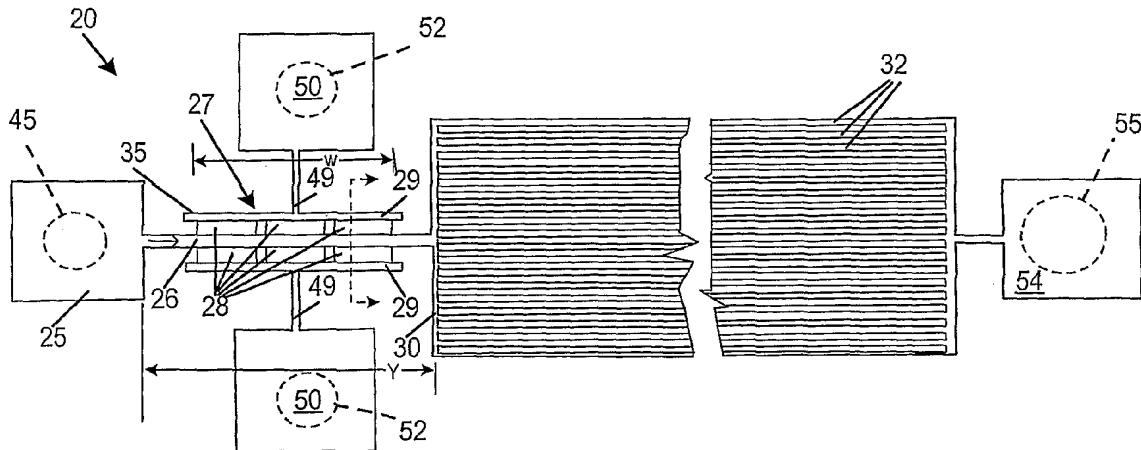
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(54) Title: INTEGRATED APPARATUS AND METHODS FOR TREATING LIQUIDS



(57) Abstract: A passive self energized, liquid device operates entirely on capillary action. A microfilter fractionates nanoliter volumes of suspension such as whole blood into suspended particles or cells and liquid fractions. Blood for example, is fractionated with minimal cell lysis, and the filtrate (plasma) flux is dependent upon design parameters similar to factors controlling blood filtration in microporous membranes, i.e. active filter area, fluid velocity and microfilter geometry. Weir-style filters communicate with a blood flow channel to separate plasma from blood moving by capillary action. An expanded downstream channel with multiple parallel capillary blood flow path provides continuing movement of blood past the filters. Lysing is controlled by the size of the filter pores and the duration of adherence of the red blood cells to the pores. The controlled lysis or prevention of lysis of red blood cells is accomplished by manipulating the significant capillary forces generated in the filters. Filtration, cell lysis and microchannel blood flow models are integrated into an overall microfilter design useful for fabricating microfilter devices for lab-on-a-chip clinical applications where they can be coupled with on-chip electrical and electro-optical devices.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTEGRATED APPARATUS AND METHODS FOR TREATING LIQUIDS**Cross Reference to Related Applications**

This application claims priority from the provisional patent application Serial No. 60/407,677 filed September 7, 2002 in the name of Vincent B. Pizziconi and Timothy Crowley entitled "Self-Energizing, Scalable and Integrative Hybrid Informatic Method and Apparatus to Manipulate, Process, Diagnose and Treat Complex Fluids" incorporated herein by reference.

Statement of Government Funding

Financial assistance for this project was provided by the U.S. Government through the National Science Foundation under Contract/Grant Number DB1 9602258. Therefore the United States Government may own certain rights to this invention.

Field of the Invention

This invention relates to microdevices and methods for treating, analyzing complex liquids and more particularly to devices of this kind formed on and integrated with semiconductor device supporting substrates.

Background**Introduction**

The potential of miniature analytical systems in medicine and biotechnology is significant and far-reaching. So called "micro total analysis systems" (μ TAS) have the potential to accelerate research in drug discovery, genomics, and proteomics by enabling very high throughput screening of complex samples. They have the potential as well to provide patient "point-of care" capability, and to permit massively parallel bioanalytical chemistry strategies. Although much progress has been made in developing the diagnostics capability of μ TAS, to date, little has been done in the way of engineering design and development

strategies to allow integrated or modular "on-chip" sample manipulation, preparation and pretreatment for complex biological fluids, such as blood and the like.

Many of the μ TAS proposed to date are based upon microfluidic transport of the sample. Microfluidics can be viewed as the physical, chemical, and biological analogue to the 5 integrated circuit. It is the manipulation of fluids on the micron and nanoliter scale using micromachined substrates comprised of complex, miniaturized fluid flow channels and control elements (e.g., baffles, protrusions, flow discontinuities, valves, heaters, pumps) to accomplish micro and nanoscale transport functions in an integrated unit operations process format. Miniaturized chemical analysis systems can reduce the need for skilled labor and 10 reagent cost, improve duty cycle time, reduce production of wastes, e.g. environmental toxins, and while potentially providing the ability to leverage unique small-scale transport effects. For health care applications, the ability to perform point-of-use miniaturization of clinical chemistry will improve patient care, minimize the need to access clinical laboratory facilities and skilled labor, and reduce costs. The use of small scale solid state device liquid 15 treatment and analysis platforms may also permit massively parallel bioanalytical chemistry strategies to be performed useful for telemedicine and other bioinformatic applications.

Microfluidic manipulation of fluids can take advantage of physical and energetic material conditions that are not possible on the macroscale. These include high surface area to volume ratios, and rapid transport processes due to small transfer distances. In addition, the 20 length scale can be designed to take advantage of unique flow behaviors of complex fluids.

Micromachining of microfluidic elements offers a degree of dimensional control in both size and geometry that was not previously possible, providing new capabilities to create innovative biomicrodevices. These microfluidic devices can be made massively parallel to accomplish finely controlled, larger volume processes. However, to take full advantage of 25 these systems additional research and development is required to better understand physical

and related scale-dependent energetic phenomena that exist at small length scales and to leverage the unique small-scale effects associated with complex fluids interfacing with other materials.

Complex biological fluids such as blood, cell cultures, and other tissue fluids represent an important class of fluids pertinent to miniaturized chemical analysis. These complex fluids typically require sample preparation to fractionate various fluid components that either interfere with chemical analysis, or provide fluid sub-fractions containing desired analytes. For example, a key operation in the analysis of blood chemistry is the separation of plasma from whole blood. Microdevices engineered with integrated, upstream sample preparation permit direct sample delivery, i.e., blood from a finger stick, and eliminate the need for additional preparatory steps. To date this capability has not been developed, and fundamental design and process research in the area of microfluidic sample preparation is needed to support the commercialization of fully integrated lab-on-a-chip devices (Lichtenberg et al., 2002).

The development of miniaturized devices for the clinical analysis of blood and body fluids is an important application of microfluidic technology. Filtration and separation of blood at the macroscale is typically performed with microporous membranes (membrane plasmapheresis) or using centrifugal techniques. U.S. patent Nos. 5,922,210 and 6,387,290 to Brody et al. issued on July 13, 1999 and May 14, 2002 and entitled "Tangential Flow Planar Microfabricated Fluid Filter and Method of Using Thereof" and "Tangential Flow Planar Microfabricated Fluid Filter" describe a liquid specimen treating arrangement that moves liquid along a narrow tube by capillary action from an input reservoir to an output reservoir. Filtration at the microscale is proposed with a weir-style filter. These patents are incorporated herein by reference. Moorthy (2003) teaches filtration of dilute suspensions of isotonically swollen blood cells using in situ fabricated microporous membranes. The utility

and cost benefits of miniaturized clinical chemistry to improve patient care has already been demonstrated with the point-of-use blood glucose monitoring systems that dramatically improve diabetic glucose control (Sonksen et al., 1978, Walford et al., 1978). Similar benefits are expected with the development of highly integrated lab-on-chip devices with the 5 capability to measure multiple blood analytes from a single drop of blood.

To date, microfluidic research applied specifically towards the needs of clinical chemistry has been limited. This is needed to develop the practical, enabling platforms required to realize these miniaturized clinical devices. These include miniaturized optical detection schemes, nanoliter chemical delivery, microscale mixing, and, in blood work, 10 microfluidic separation of plasma from whole blood.

As for cell lysis, where that, rather than just filtration is desired, lysis of blood for microscale applications has been proposed using chemical lysing agents. Mechanical lysis using externally applied energy (pressure) and through the use of electric fields has been described. No known prior art has proposed the lysis of cells using only capillary action for 15 microscale operations that are thus "passive." As used herein "passive" means operable without the application of an external force or energy as in the case of movement of a liquid solely by capillary action.

In the embodiment of the present invention, the level of stress applied to cells can be very precisely controlled using passive surface energy and precise geometrical 20 structures. Various levels of lysis may be achieved using this technique. With the present invention, using specific filter parameters, geometry, etc. is believed possible to selectively lyse specific cell types while other cells are not lysed, e.g. white blood cells vs. red cells.

Characterization of rheological properties of complex fluids is traditionally done with sophisticated rheological equipment, e.g. cone/plate viscometer or rheometer equipment. The 25 devices are typically bench top instruments requiring external energy, sophisticated

mechanisms and equipment to measure the rheological properties of complex fluids and volumes on the order of milliliters. Again passive microscale devices suitable for lab-on-a-chip use are desirable according to embodiments of the present invention.

Brief Summary

5 A passive, self-energizing small (micro-nano) scale liquid treatment and analytic microtool according to this invention uses capillary action to move and manipulate a liquid specimen. The specimen may be a single sessile drop of the liquid specimen being
manipulated and tested.

In a preferred embodiment, a flow channel in which the liquid specimen moves by
10 capillary action from an input reservoir leads to an expanded channel. There, parallel liquid paths connected to the flow channel sustain the presence of the motivating force and thus duration of capillary action moving the liquid in the flow channel. This extends the device operating time. A comparable in length flow channel without the expansions described here is estimated to be able to operate for about 15 seconds, whereas with this expanded channel
15 here described operation times up to two minutes are possible. Filters opening from the flow channel direct liquid of desired specimen subfractions filtered from a specimen to a collection region. Again capillary action is the motivating force. The arrangement is particularly useful in the fractionation of a complex fluid suspension such as blood, where suspended matter such as macromolecules and formed elements (e.g. red and other blood cells) are moved
20 along the liquid flow channel and while a desired subfraction of filtrate such as plasma exits via the filter constructs into the collection region. Plasma production is in the nanoliter range compared to the picoliter of the prior art "straight channel" device.

In one exemplary embodiment the liquid specimen or filtrate from the specimen is moved along a liquid flow channel by capillary action and in accordance with the invention
25 to locations where portions of the liquid are tested or examined. Liquid testing apparatus is

provided to test the liquid in each of the wells. Optical, chemical, biological and electrical testing may be carried out on the liquid with or without reagents.

Broadly, in accordance with one aspect of the invention the method of liquid treatment and analysis comprises devising a set of heuristic design rules from the behavior of 5 nanoliters of a particular liquid, fabricating as a part of a liquid treatment and analyzing instrument a liquid contacting device based on one or more of the heuristic rules and providing a means of determination of a characteristic of the liquid based on the liquid's behavior in contact with the liquid contacting device. Process models and design conditions have been established that permit the a priori design of microfilter device to meet specific 10 design constraints.

In the case in which the liquid comprises a suspension of particles, such as colloids and cells, devising heuristic rules includes determining parameters of the liquid contact device that affect lysis of the cells, and the fabricating of the liquid contacting device includes implementing selected values of the parameters to provide the desired presence or absence of 15 lysis of the cells. A particular preferred and exemplary embodiment of this method is applied to blood fractionation in which blood cells, such as red blood cells, suspended in plasma are the cells and the parameters include cell stress and cell stress duration.

An instrument made pursuant to the heuristic rules in accordance with the invention in one embodiment includes a liquid input opening, a first passage leading from the liquid input 20 opening and operative to move liquid therealong by capillary action, a filter communicating between the first passage and a filtrate collection location to which the filtrate flows, again by capillary action, and an expanded liquid flow path for passing the liquid from the first liquid passage to a further location. The expanded liquid flow path preferably comprises a plurality 25 of liquid flow paths connected to receive liquid flow in parallel from the first passage and sized to move the liquid therein by capillary action. In this flow network arrangement, the

multiple parallel capillary action paths sustain the movement from the input location through the first passage in communication with the filter for a longer period of filtration and collection of a higher quantity of filtrate than if only the single, first passage were employed.

In another exemplary embodiment of the invention, controlled lysis of cells in a suspension such as red blood cells in liquid whole blood is accomplished. Again, filter openings or pores communicate through the sides of a liquid flow channel along which the suspension moves by capillary action. The size of the pores and the length of a passage from the pore that draws liquid to a collection site are chosen to provide controlled levels of cell stress and duration and thus control the level or prevention of lysis of cells that adhere to and are deformed by the filter opening. The factors that affect lysing are stress on the cell and the duration of such stress. In the case of fractionation of a suspension such as whole blood, where only liquid such as plasma is desired as the filtrate, then the filtrate flow channel away from the filter to the filtrate collection site is made sufficiently short as to shorten the time of residence of a cell at the filter pore particularly during the initial wetting period when capillary pressure across the filter pore or pores is greatest. On the other hand, if a greater or lesser degree of lysis is desired, then, a longer channel is used.

An exemplary instrument for measuring capillary pressure is a part of this invention. An encapsulation of entrapped gas, air for example, is provided proximate a flow of liquid by capillary action. A tube from the path of liquid flow to the gas develops a column of the liquid that acts against the gas to an extent that is indicative of the capillary pressure present. The location of the meniscus of the liquid column is detected as an indicator of the capillary pressure. A pair of these pressure measuring arrangements spaced apart in the direction of liquid flow can serve as a flow meter.

Where the liquid under examination is clear (i.e. transparent), a preferred method of illuminating the liquid allows its observation. A substrate is provided on which an at least

partially light transmitting layer is present and forms with the substrate an interface. The liquid specimen is supported on the surface of the at least partially light transmitting layer. Because of differences in indices of refraction, the critical angle of reflection at the surface of the at least partially light transmitting layer changes. This permits visible interference
5 between light reflected from and light refracted through the layer to be altered or eliminated so that the presence of absence of a liquid on surface of the layer is more readily observed. A specific exemplary embodiment of the method and device employs a silicon substrate on which a silicon dioxide layer has been grown to serve as the at least partially light transmitting layer of the device.

10 In particular exemplary embodiments of the invention a plurality of specimen treating devices of the kind described above have been integrated into a single semiconductor chip. As many as eight filtration units have been formed on the chip. Preferably the units share at least one vent line connecting filtrate collection regions and outputs of the flow channels to at least one opening to atmosphere. The expanded flow channel with parallel capillary flow paths can be prepared such that the parallel capillary flow paths are formed in a serpentine pattern so as to be fit, with other such parallel capillary flow paths, on the single semiconductor chip. Additional features carried by the chip include passive hematocrit measuring arrangements for moving blood by capillary action through a serpentine path. The distance the whole blood moves is a measure of % hematocrit.
15

20 A filtration and separation method is provided that is adaptable to microscale technologies. Neither membrane plasmapheresis nor centrifugation is readily adaptable to lab-on-a-chip type applications. The techniques required to fabricate membrane type technology in microchannels is not well established. Additionally, the use of centrifugation at the microscale is problematic for the following reasons: 1) g-forces achieved in
25 centrifugation are related to the angular velocity and the radius of rotation. The radius of

rotation in microdevices is small and very high rotation rates, >15000 rpms, are required to achieve the g-forces required to separate biological fluids such as blood. 2) rotation requires additional mechanical equipment and a power supply. The passive devices of this invention do not require any external energy or equipment. The performance of the proposed device as measured on a filter flux basis is significantly higher, 3-5x higher than membrane plasmapheresis.

Typically the liquid volumes being treated are of the nanoliter size. Ordinarily, a single drop of the liquid suffices for the purposes of the invention. In one exemplary embodiment the liquid flow channel that draws the liquid past the weir-like filters is of a cross-sectional dimension less than $10\mu\text{m}$ and greater than $0.1\mu\text{m}$. Preferably the dimension is about $0.5\mu\text{m}$.

The devices can be used to measure the rheological properties of complex fluids without external energy sources. They do not require sophisticated electronic and mechanical equipment. Measurement is made simply by measuring the distance the fluid traveled in specifically designed microchannels. These devices are suitable for having the measurement and readout performed by an optical assessment of the fluid position using only, for example, a CCD camera. Using a micromachining device can achieve very finely controlled geometrical and flow conditions that are not achievable with typical macroscale devices.

An exemplary embodiment of the method of fabricating the liquid specimen handling devices of the invention uses techniques similar to those used in the fabrication of integrated circuits. A photoresist is deposited on a semiconductor substrate. A mask defining the liquid flow provisions of the device is brought into place and the photoresist is exposed to UV light through the mask. The photoresist is removed in locations that have been exposed to the UV light. A semiconductor is etched in areas revealed by the removal of the photoresist to form the liquid flow provisions and other features of the device. A closure layer, typically a glass

lid, is secured to the semiconductor substrate over the etched channels and other device features. Where weir-like filters are to be prepared, either before or after forming the liquid flow channels, just one further mask defining filter pores is brought into alignment with the semiconductor separate and aside from the foregoing steps and the procedure of exposing a 5 photoresist on the surface of the semiconductor to UV light is used in relation to just the areas that are to be the pores of the filter. The exposed photoresist areas are removed and the pores are etched to a depth that is less than that to which an adjacent flow channel is etched.

The above and further features and advantages of the invention will be better understood with reference to the following detailed description of preferred embodiments 10 taken in consideration with the accompanying drawings. The illustrations in the various Figs. of the drawings are for explanatory purposes, and unless otherwise noted, are diagrammatic and not to scale.

Brief Description of the Drawings

Fig. 1 is a diagrammatic illustration of a microfilter formed on silicon in a manner 15 similar to semiconductor fabrication;

Fig. 2 is a diagrammatic cross section along the lines 2-2 of Fig. 1 and shows a pair of weir-like filters opening from a main fluid channel to two filtrate receiving side channels;

Fig. 3 is a diagrammatic illustration of three microfilters sharing flow-enabling vent 20 lines on a single silicon chip;

Fig. 4 is a diagrammatic, enlarged cross-sectional view of a single weir-like filter having a red blood cell adhering to its input opening;

Fig. 5 is a diagrammatic illustration of the steps in the formation of a fluid handling device like the filter of Fig. 1;

Fig. 6A - 6C are diagrammatic illustrations of a multi-channel analysis chip with 25 electro-optical test provisions;

Fig. 7 is a diagrammatic illustration of a series of filters of varying lengths in communication with a blood flow channel;

Fig. 8 is an enlarged fragmentary illustration showing one of the filters of Fig. 7;

Fig. 9 is a diagrammatic illustration like Fig. 7 of a series of filters of varying lengths
5 in communication with a blood flow channel;

Fig. 10 is a capillary pressure gauge having entrapped air acted on by a column of fluid;

Fig. 11 is a schematic illustration of a flow meter made up of two of the capillary pressure gauges of Fig. 10;

10 Fig. 12 is a schematic illustration of an optical arrangement for viewing otherwise transparent fluid moving on a surface;

Fig. 13A is a top plan view of a silicon-based integrated fluid device incorporating eight filters and a pair of hematocrit meters on a single silicon chip;

15 Fig. 13B is an enlarged fragmentary plan view of the device of Fig. 13A and showing a series of parallel, serpentine capillary flow paths;

Fig. 14 is a diagrammatic illustration of anodic bonding equipment used to bond a glass closure or lid onto a silicon substrate.

Fig. 15 is a plot of individual percent red pixel values for hemoglobin reference standards vs. hemoglobin concentration;

20 Fig. 16A and B are plots of hemoglobin concentration and plasma filtrate vs. filter pore width showing, in A, individual results, and in B, average of three tests per point with filter pores 200 μm long;

Fig. 17 A and B plots hemoglobin concentration and plasma filtrate vs. filter pore length at three pore heights showing individual results: A an average of three tests per point,
25 and B with filter pores 500 μm wide;

Fig. 18 is a plot of plasma filter flux vs. pore length for three different pore heights;

Fig. 19 is a plot of plasma filter flux vs. pore width for three different pore heights;

Fig. 20 is a plot of plasma flux vs. pore height for all individual microfilter tests and

average flux at each value of pore height;

5 Fig. 21 is a plot of hemoglobin concentration in plasma vs. time for plasma to cross
the filter;

Fig. 22 is a plot of time to hemolysis vs. membrane tension as developed by Rand
(1964) and Blackshear and Anderson (1977);

10 Fig. 23 is a representation of a model of red cell deformation into a rectangular
microfilter pore, which model is used to estimate membrane tension;

Fig. 24 is a plot of modeled membrane tension vs. pore height for red cell deformation
in rectangular pores via capillary action;

Fig. 25 is a plot of average hemoglobin concentration vs. pore height squared for all
filter width tests;

15 Fig. 26 is a plot of average experimental flux at three filter pore heights vs.
residence time model of flux with error bars showing the range of experimental flux data;

Fig. 27 is a plot of plasma flux vs. wall shear rate as previously described for
membrane plasma pheresis and as observed for microfiltration of the experimental model
according to the invention;

20 Fig. 28 is a diagrammatic illustration of a generalized microfilter in accordance with
the invention and lists parameters affecting the design of the filter;

Fig. 29 is a plot of actual plasma volume vs. modeled plasma volume for a microfilter
in accordance with the present invention;

Fig. 30 is a plot of expanded channel width vs. filtration channel length with shadings
25 indicating plasma volume from a microfilter in accordance with the invention; and

Figs. 31A and 31B are plots of expanded channel width vs. filtration channel length first without (A) and then with (B) limitations imposed by wall shear rate limit and hematocrit design rule limitation.

Detailed Description

5 Microfilter

As illustrated in Figs. 1 and 2, a microfiltering system 20 is fabricated in silicon and glass. This is done using methods similar to those used in integrated circuit fabrication as described below. The microfilter 20 has an input reservoir 25, a narrow filtration channel 26 containing a pair of transverse flow microfilters 27, a pair of plasma outlet channels 29 to collect filtrate. A downstream expanded channel 30 connects in series with the filtration channel 26. In one exemplary embodiment, the expanded channel 30 of the device contains an array of parallel, $\sim 45\mu\text{m}$ wide, and 15mm long flow channels 32, as shown in Fig. 1.

The microfilter 27 is a weir type filter fabricated as a series of rectangular openings 28, the 'filter pores', located on both sides of the $100\mu\text{m}$ wide filtration channel 26. In one preferred exemplary embodiment, used for the fractionation of nanoliter amounts of whole blood, the dimensions of each filter pore was a width 'w' $200\mu\text{m}$ wide, a height 'h' $<1\mu\text{m}$ high, and a length 'l' $\sim 30\mu\text{m}$ long. The $\sim 30\mu\text{m}$ length represents the length 'l' between the blood flow channel 26 and a plasma outlet channel 29 as illustrated in Fig. 2. The total $200\mu\text{m}$ width 'w' of the active microfilter section is approximately 75% of the filtration channel length 'y'. Also, the pores 28 of the filter were centered over the length of the filtration channel 26.

As shown in Fig. 2 the features of the microfilter are formed in a Si substrate 40 and are covered over by a glass lid 42. The broken line circle in Fig. 1 represents a hole 45 bored into the glass lid 42 above the input reservoir 25 for the introduction of a drop of liquid to be separated or in the case of blood "fractionated." In the case of whole blood, red blood cells

47 are greater in cross-sectional dimension than the height of the pores 28. Plasma, the filtrate, is delivered to plasma outlet channels 29. From there the filtrate is drawn through a pair of narrow channels 49 by capillary action to a pair of reservoirs or collection regions 50. As indicated by the broken line circles at the reservoirs 50, a pair of further holes 52 vent the 5 reservoirs to atmosphere to permit filtrate flow to the reservoirs.

Capillary action in the filtration channel 26 initiates the flow of specimen liquid therein and capillary action in the flow channels 32 of the expanded channel 30 continues to draw the specimen liquid into and along the filtration channel 26. In the case of blood the red cell rich suspension in the parallel channels 32 is delivered toward output reservoir 54 that is 10 also vented to atmosphere by a hole 55 in the glass lid or closure 42.

It was determined that plasma flux across the filter is substantially independent of the % hematocrit of the specimen. This is an unexpected and beneficial result for clinical use of such microfilter device, making their performance very much independent of % hematocrit variances among individuals or within an individual.

15 In the microfilters as described, generally, the red cell rich (high % hematocrit) flow in the expanded channel would not reach the end of the parallel capillary flow paths. This arose either by blocking of the vent line or by stoppage of the viscous sludge-like blood flow high in red blood cell content by virtue of the filtering off cell free filtrate subfractions, such as plasma.

20 Two approaches help to sustain the movement of liquid in the flow channel. First, increase the available surface energy to sustain capillary action by extending the effective length cross-section, and aspect ratio of the blood flow path and thus surface area in the expanded channel. This can be achieved by making the extended flow channel longer, increasing the surface roughness of the flow channel, changing the cross-sectional area,

length to height to width ratios, or extending the 2-D planar flow channel into higher dimensions, i.e. 3-dimensions (x,y,z). These conditions will allow the desired level of shear rate for a desired amount of extraction of filtrate subtraction to be obtained. For example, just narrowing the expanded channel will increase the velocity of flow there to enhance shear rate. Second, attention to the number of pores of the filters and their size and distribution so as to control the amount of plasma transferred out of the flow will reduce the tendency of premature blood flowing in the expanded channel. Limiting plasma reduction to 10% or less is desirable to these ends.

Single Chip Multi-filter

Two different multi-microfilter device designs are referred to as 'Weir 10' and 'Weir 11'. The design of the flow channel network and microfilter barrier geometry is identical between the two designs. However, the total width and resultant surface area of the expanded channel, and the layout of the plasma outlet channels are slightly different. The total width of the expanded channel area (i.e. the total number of individual parallel channels) was specified at a magnitude proportional to the filtration channel length to achieve an acceptable level of shear rate in the expanded channel. Table 1 provides key design features for both designs and Table 2 summarizes the Weir 10 and Weir 11 design differences.

Shown in Fig. 3 is the Weir 10 multiple filter device 60 layout integrating three microfilters 61, 62 and 63 into a single test cell sharing a common channel 65 that allows the displaced air to vent during wetting. Three input reservoirs 67, 68 and 69 feed three filter flow channels 72, 73 and 74. Three weir-style filters 77, 78 and 79 divert liquid filtrate to three sets of filtrate output flow channels 81, 82 and 83. Three expanded channels 86, 87 and 88 of differing geometry contain parallel capillary flow paths (not separately shown). At reservoirs 90 and 91, connected with the common vent channel 65, vent holes 93 and 94 are

drilled in a glass lid that overlies the features etched in Si to form the multi-filter device 60. The Weir 11 microfilter devices utilizes individual vent holes instead of the integrated vent line 65 used in the Weir 10 design. Also, the Weir 11 plasma outlet channels were designed as large, wide rectangular open areas, containing the parallel capillary flow paths, while the
5 Weir 10 devices utilizes the long narrow channel 86, 87 and 88.

Passive microfilter devices like Weir 10 (Fig. 3) and Weir 11 (not shown) operate successfully to isolate up to ~50nl of plasma from whole blood without significant levels of hemolysis. These devices effectively separate nanoliter volumes of plasma utilizing only capillary action in a design platform that supports integrated sample preparation for lab-on-a-
10 chip devices. Additionally, the microchannel layout with the expanded channel extends the passive operation time of these devices by several fold, i.e. a few seconds to several minutes.

Table 1. Key microfilter design features.

Target Shear Rate sec ⁻¹	Main Channel Length (mm)	Weir 10 Design Number of Individual Channels in Expanded Area	Weir 11 Design Number of Individual Channels in Expanded Area	Filter Length (mm)	Number of Filter Pores
5000	2	100	50	1.4	12
2500	4	50	25	2.8	24
1100	15	14	7	11.3	94

Table 2. Key design differences between Weir 10 and Weir 11 microfilter designs.

Design Feature	Weir 10	Weir 11
Total Width of Expanded Channel	2 times larger than Weir 11	½ size of Weir 10
Expanded Channel Target Shear Rate (s^{-1})	100	200
Device Layout on Wafer	Three devices integrated into a common test cell.	Devices laid out individually across wafer
Venting	Integrated vent line shared by three devices	Individual venting for each device
Plasma Outlet channels	Long narrow channels ~60um wide	Large rectangular collection areas 3mm wide

Multi-Channel Analysis Chip

As shown in Fig. 6A - C, a generalized bioanalytical microdevice 100 illustrates the potential impact of microfluidic research on clinical chemistry. This device is a "micro sequential multi-channel analysis chip" (μ SMAC) and has the capability to perform, at 16 locations 102, 16 different blood tests from a single drop of blood. This disposable test cartridge 100 contains microfilters, shown diagrammatically in Fig. 6B at 103 for the separation of plasma, plasma collection chambers 104 for chemical analysis, and optical detection cells 106, Fig. 6C, for quantification of the analytes. Analysis is initiated by placing a drop of blood from a finger stick on the inlet reservoir 107. As blood flows through the device via capillary action, plasma is separated by the microfilters located on each of the sixteen analysis channels 109. Each plasma outlet chamber 104 contains analysis reagents Fig. 6C and forms a horizontal optical detection cell with a 2 mm long optical path length, requiring only nanoliter volumes of plasma. The test cartridge is evaluated using a handheld device, similar to commercial glucose test systems, containing the optical and electrical systems required to quantify the test results. These may include a laser 110 and a

photodetector 112, for example, viewing the analyte through the glass cover 114 of the device using mirrored sidewalls 111 of the optical detection cell. With large-scale integration of microfluidic and microelectronic devices, it becomes possible to integrate all these functions into a stand-alone test cartridge. As nanotechnology progresses, it is believed 5 it will be possible to develop a fully implantable μ TAS system. In any case, the realization of such a device has broad reaching implications for patient care and the availability of sophisticated clinical analysis to the general populace.

Using the design rules and the operation model described herein, a multi-channel microfilter device 115 was designed and fabricated as shown in Figs. 13A and 13B. This 10 device has the capability to isolate blood plasma in eight separate microchannels 123 (Fig. 13B). The device includes, on a single Si chip 116, eight integrated expanded channel microfilter devices connected to a common input reservoir 118 and utilizing a common air vent line 119. It also uses ~8mm long filtration channels 117 with 10-channel expanded channel sections 121 utilizing a serpentine flow channel layout best illustrated in Fig. 13B.

15 Also included in the multi-filter construction of Fig. 13A are two integral hematocrit test devices or gauges 122. In these a single capillary flow channel 124 leads from the common input port 118, through a series of flow retarding chambers 125 to a single serpentine section 126 of the channel 124. The channel 124 is vented, at the far end of its serpentine section by the common vent line 119.

20 The distance along the single channel 124 that the whole blood from the specimen travels by capillary action is a function of the % hematocrit of the specimen introduced to the reservoir 118.

25 Preliminary testing of the device was performed using whole bovine blood. Blood was observed to flow uniformly through all eight filtration and serpentine structures. Also, plasma was successfully isolated in all 8 plasma outlet channels and the μ SMAC prototype

device demonstrated that blood could be fractionated using a multichannel microfilter design. These preliminary results support the feasibility of developing massively parallel multi-channel blood analysis devices for *in vitro* clinical chemistry applications, biotechnology, and potentially *in vivo* applications.

5 Microfilter Design Heuristics

Based on the tests described below, the following heuristic rules were determined:

- The control of hemolysis is a key design specification for lab-on-a-chip applications. The filter pore geometry, i.e. pore height h , was determined to be a key factor. Hemolysis can be minimized in microfilter design through the use of pore heights $<0.5\mu\text{m}$ or some equivalent pore diameter, D_e .
- Filter pore length l was found only to have a secondary effect on hemolysis, and can be limited by minimizing the filter pore length.
- The filter pore residence time model of hemolysis, developed in this study, was qualitatively in good agreement with the experimental results, i.e. larger filter pore heights permit greater deformation of the red cell 47 into the filter pore (at 125 in Fig. 4, for example) than smaller filter pores.
- Hemolysis is increased with filter pore geometries that increase residence time of the cells in contact with the filter pore whereupon red cells are subjected to the significant capillary pressures present upon initial wetting of the filter for a longer period of time.

Microfilter Cell Lysis Background

Cell lysis, e.g. red blood cell hemolysis, can occur in microfilter devices due to the deformation of red cells into sub-micron sized filter pores, and the membrane stresses generated by the transfilter pressure, as shown in Fig. 4. In capillarity-driven microfilters, the highest transfilter pressure is generated upon initial wetting of the sub-micron filter pore by

capillary action when capillary pressure is generated by plasma 127 initially crossing the filter, after which it decreases when fluid reaches the deeper plasma outlet channel where the capillary pressure is reduced. The apparent capillary pressure generated in the filter pore can be substantial, and it is at least an order of magnitude higher than the pressure generated by
5 the outlet channel.

Hemolysis, and cell lysis in general, is a time-dependent function of stress, i.e. hemolysis occurs more quickly at high stress and slowly at low stress. Alternatively, hemolysis can be prevented at high stresses if the exposure time is very short, and at longer exposure times if the stresses are lower. In microfilter devices, the duration and level of
10 stress generated by filter wetting is related to the filter pore dimensions of length, width, and height. Filter length controls the time that the high capillary pressure, generated by filter pore wetting, is present at the filter face, as this parameter determines the length of time for plasma to cross the filter and reach the deeper plasma outlet channel. The filter pore width and height affect the capillary pressure generated across the filter barrier. Also, these parameters can
15 affect the shape of the red cell deformation or the residence time of the cell in the filter. As such, hemolysis experiments were conducted to investigate the effects of pore length, height and width as these parameters control the duration and level of stress applied to red cells.

Hemolysis Test Devices

Methods

Microfluidic separation devices designed to assess factors controlling hemolysis were fabricated in silicon and glass with the techniques of the present invention. Test structures 5 were developed to characterize the effects of filter geometry on hemolysis. On-chip colorimetric techniques were also developed to assess the level of hemoglobin in the nano-liter volumes of plasma filtrate as a method to quantify hemolysis. Hemolysis test devices varied the pore length, width, and height over the experimental range shown in Table 2. Two basic hemolysis test device designs were utilized, one device 130 was designed to vary the 10 filter lengths l as shown in Fig. 7. The second test device 150 was designed to vary the filter width w as shown in Fig. 9. Both test devices were fabricated at three different pore heights h . The blood flow channels were $100\mu\text{m}$ wide, and plasma outlet channels were $60\mu\text{m}$ wide.

In Fig. 7A blood plasma is introduced at a reservoir 131 and travels by capillary action through a flow channel 133 to an output reservoir 135. Along the way the blood 15 plasma passes a series of the weir-style filters 137 of varying lengths. Plasma and any lysed cell is carried by capillary action of tubes 139 to a collection region 141. The tubes 139 and the collection region 141 are vented at 142. Fig. 8 is an enlarged illustration of one of the filters 137 in association with the flow channel 133 and the tube 139 therefrom. Two pores 143 and 144 are shown. Each has a length l and a width w .

20 Similarly, Fig. 7B illustrates a hemolysis test device of variable filter width w . It has an input reservoir 151 from which blood flows along a flow path 153 to an outlet reservoir 155. Filters 157 have varying pore widths w . The core width w is illustrated in Fig. 8. Again tubes 159 carry plasma by capillary action to a collection region 161 vented to atmosphere at 162.

Hemolysis testing was performed at room temperature by applying approximately 10 μ l of citrated whole bovine blood to the input reservoir of each test device using a micropipette, after which blood flowed spontaneously through the device via capillary action. After blood ceased, the plasma filtrate produced by each microfilter was digitally photographed at 100x using a Mitutoyo Ultraplan FS110 optical microscope (Aurora, IL), and a JVC TK-1280U color video camera (Cypress, CA). The concentration of hemoglobin in the plasma filtrate was measured using a colorimetric comparison, between the plasma filtrate and four hemoglobin reference standards, 0, 1.7, 3.3, and 6.7 g/dl, incorporated into each wafer. The colorimetric assessment of hemoglobin standards and the plasma filtrate was based on digital color analysis of the hemoglobin and isolated plasma using the red, green, blue (RGB) color model. The redness of the hemoglobin standards and plasma filtrate was quantified by calculating the average percent red pixel value per equation the following equation for % Red. A correlation between percent and hemoglobin concentration was established using the on-wafer hemoglobin reference standards and utilized to quantify the hemoglobin concentration in isolated plasma.

$$\% \text{Red} = \frac{\text{Red Pixel Value}}{\text{Red} + \text{Green} + \text{Blue Pixel Values}} \quad (1)$$

Hemoglobin standards were prepared from citrated bovine blood. The isolated red blood cells were washed. Red cells were hemolyzed. The isolated hemoglobin solution was then diluted with DI water to produce three solutions containing 1.7, 3.3, and 6.7 g/dl hemoglobin as determined by absorbance measurements. Water was used as the zero hemoglobin reference. The hemoglobin reference solutions were placed in specific microfluidic channels on each wafer and sealed with epoxy to prevent evaporation.

The volume of plasma fractionated by each filter was calculated using digital analysis of optical micrographs. The area of the outlet channel filled with plasma was first measured,

and multiplied by the known channel height to calculate plasma volume. Plasma flux for each filter was then calculated by dividing the plasma volume extracted by the filter area and time of operation.

Hemolysis testing and evaluation was performed on 115 individual microfilter test
5 structures. The volume of plasma and the concentration of hemoglobin in the plasma was quantified for each device and compared to filter parameters of pore height, width, and length. Test devices were fabricated on three silicon wafers, each wafer included five filter length and five filter width test devices (Figs. 7A - 7B). Each wafer was fabricated at one of three pore heights, 0.37, 0.67, or 1.28 um. The average channel height of the blood flow and
10 plasma outlet channels was 21um.

Bovine blood specimen with a hematocrit of 38.5% was used for the hemolysis testing. Results for hematocrit, viscosity, total protein and fibrinogen concentration were consistent with other bovine blood specimens used in this research.

The four hemoglobin standards on each test wafer were photographed before and after
15 device testing and digitally analyzed to determine the degree of red color, calculated as %Red pixel value. Fig. 15 shows all measured values of %Red for the four hemoglobin standards on all wafers. The hemoglobin concentration in the isolated plasma of each microfilter was determined based on the %Red pixel value. Three microfilters were tested at each level of pore height, width and length, and the average hemoglobin concentration was calculated. The
20 hemoglobin concentrations versus pore height, width and length are shown in Figs. 16 and 17. Additionally the effects of pore dimensions on plasma filtrate flux are shown in Figs. 18 - 20. The average plasma flux was calculated based on an estimated operating time of 10 seconds for all devices, as the experimental methods were not capable or intended to measure blood flow rate and device operation time for each test.

An optical micrograph of a hemolysis test device with isolated plasma was taken from a test device with the largest pore height ($1.28\mu\text{m}$). In these $1.28\mu\text{m}$ pore height devices cells were observed to penetrate the filter area, and in some devices a few cells could be observed in the plasma filtrate.

5 Discussion

The effects of microfilter height, width, and length, on hemolysis were based on a colorimetric assessment of hemoglobin concentration in the extracted plasma. This on-wafer colorimetric measurement technique was originally developed as part of this research. The colorimetric assessment was calibrated using on-wafer hemoglobin standards as shown in Fig. 15. The correlation between % Red and hemoglobin concentration in the standards was evaluated for statistical significance using Anova, and the difference was found to be highly significant, $p < 0.005$. This correlation demonstrates the validity of this colorimetric technique to measure hemoglobin in the isolated plasma.

The experimental data indicate that pore height and length effect hemolysis in microfilters while pore width was not significant (Figs. 16 - 17). Pore height, or an equivalent De, was the primary modulator of hemolysis with larger pore heights producing higher levels of hemolysis. As can be observed in Fig. 17, the effect of pore length was modulated by pore height, as hemolysis was not sensitive to filter length at the smallest pore height. From a design standpoint, the data indicate that hemolysis is minimized at pore heights less than $0.5\mu\text{m}$, and devices should be fabricated with the minimum filter length achievable by the fabrication process. The minimum pore length attainable using the equipment and techniques utilized in this study is approximately $20\mu\text{m}$.

To consider the physical mechanisms responsible for hemolysis in microfilters, the effects of filter geometry on membrane stress and time at stress must be considered. As discussed previously, filter length can modulate the time required for plasma to cross the

filter pore and reach the deeper outlet channel. During this time, stress on red cells at the blood-filter interface is highest due to the high capillary force generated by the filter pore. Once plasma reaches the outlet channel, stress on the red cells is greatly reduced, as the capillary pressure generated by the outlet channel is more than an order of magnitude less
5 than the filter pore. In the experimental devices, this time was estimated by assuming that the filter pore must be completely filled with plasma before wetting the outlet channel. The time for plasma to cross the filter was estimated per the following equation, where t is the time to cross the filter, H, W and L are the filter pore dimensions of height, width and length, and J is the average plasma filter flux.

$$10 \quad t = \frac{\text{PoreVolume}}{\text{Flux} * \text{PoreArea}} = \frac{H * W * L}{J * H * W} = \frac{L}{J}, \quad (2)$$

where t is the time to cross the filter, H, W and L are the filter pore dimensions of height, width and length, and J is the average plasma filter flux.

Using this equation, the time required for plasma to cross the filter was calculated for the filter length test devices and compared to the concentration of hemoglobin in the filtrate,
15 as shown in Fig. 21. The time required for plasma to cross the filter varied from a few hundred milliseconds up to ~ 4 seconds. Filter wetting time was similar among all test devices, and the effect of pore height on hemolysis cannot be attributed to differences in filter wetting time. However at large pore heights the level of hemoglobin in the plasma filtrate increases with time, and this result demonstrates that filter pore length has a secondary effect
20 on hemolysis.

The other factor that controls hemolysis, membrane stress, was initially evaluated for the experimental devices following the approach of Rand, previously discussed in the theory section. In Rand's study the portion of the red cell entering the filter was treated as a hemisphere, and the membrane stress was estimated by equation σ_m , where σ_m is the

membrane tension, h_p is half the pore height and the radius of the hemispherical portion of the cell in the filter, R_c is the radius of the cell outside the filter, and ΔP is the transfilter pressure. Assuming the transfilter pressure is equivalent to the capillary pressure generated in the filter by plasma, the pressure is calculated per equation where σ is the surface tension of plasma to air. For these conditions, $1/R_c$ is small compared to $1/h_p$ and equation σ_m can be reduced to equation. Due to the interaction between pressure, pore height, and membrane stress, the pore height term cancels. This equation predicts that membrane stress is equal in all devices with a membrane tension on the order of ~35 mN/m. On the basis of the models and studies of Rand, and Blackshear and Anderson (Fig. 22), this is a very high level of membrane stress, and hemolysis should occur nearly instantaneously.

$$\sigma_m = \frac{\Delta P}{2\left(\frac{1}{h_p} + \frac{1}{R_c}\right)} \quad (3)$$

$$\Delta P = \frac{\sigma}{h_p} \quad (4)$$

$$\sigma_m = \frac{\sigma}{2} \quad (5)$$

Since Rand's methodology does not explain the effect of pore height on hemolysis observed in this study, the approach was modified to take into consideration the rectangular shape of the microfilter pore. In Rand's approach, the cell was assumed to deform into the filter pore with a hemispherical shape. This assumption is reasonable for a circular pore, but with rectangular pores, that are much wider than a single cell, this condition is unlikely. An alternative approach is to assume that cells deform into the filter pore with a disc shape as shown in Fig. 23. With this assumption, the membrane tension can be estimated per equations (6,7,8), where $P_2 - P_1$ is the transfilter pressure. The dimensions of the deformed

cell are estimated by assuming that the area and volume of the cell remain constant up to the point of hemolysis. The portion of the cell outside the filter is estimated as a sphere, and the portion of the cell in the filter is estimated as a semicircular cylinder. Using the values of cell area and volume reported by Skalak et al (1973), surface area 147 μm^2 and volume 92 μm^3 ,

5 the cell dimensions and membrane stress were calculated as shown in Table 3.

$$P_3 - P_1 = \sigma_m \left(\frac{1}{h_p} + \frac{1}{R_d} \right) \quad (6)$$

$$P_3 - P_2 = \frac{2\sigma}{R_c} \quad (7)$$

$$P_2 - P_1 = \sigma_m \left(\frac{1}{h_p} + \frac{1}{R_d} - \frac{2}{R_c} \right) \quad (8)$$

Table 3. Membrane stress analysis based on cell deformation as a disk.

Pore Height (μm)	Cell Radius (μm)	Disk Radius (μm)	Membrane Tension (mN/m)
0.2	2.789	3.7188	58.6
0.4	2.777	3.7184	61.6
0.6	2.765	3.7182	64.8
0.8	2.752	3.7181	68.5
1	2.740	3.7182	72.8
1.2	2.728	3.7185	77.6

10 This analysis indicates that membrane stress increases with increasing pore heights (Fig. 24). However, as with the previous analysis, the estimated membrane stresses are very high, which suggest that hemolysis should occur on a millisecond time scale in all devices. Neither model of membrane stress could account for the experimental dependency of hemoglobin concentration on pore height as observed.

15 In a study of hemolysis for membrane plasmapheresis, Zydny and Colton (1982) proposed that red cells occlude filter pores momentarily and then dislodge due to drag forces or interactions with other cells. They hypothesized that the residence time of a red cell in the filter pore was inversely proportional to shear rate, and therefore hemolysis was reduced at

higher shear rate. Using this approach to investigate the correlation between pore height and hemolysis observed in this study, cell residence time is hypothesized to be a function of the area of the cell embedded in the filter pore and shear rate. The area of the cell in contact with the filter generates a “frictional” force that holds the cell in the filter, and shear rate at the 5 filter modulates the release of the cell through drag forces or cell-cell interactions. The portion of the cell protruding in the filter is assumed to take on a disk shape with radius R_d , related to pore height by equation (9) where k_1 and k_2 are constants.

This equation predicts that the deformation of a cell into a filter pore reaches a minimum as pore height decreases. The area of cell in contact with the filter, $A_{contact}$, can be 10 computed as shown in equation (10). Residence time of a cell, t_r , is proposed to be a function of shear rate and contact area as shown in equation (11), where c and k are constants. Under this model residence time of a cell in the filter is related to the square of the pore height, and shear rate as shown.

$$R_d = k_1 + k_2 h_{pore}^2 \quad (9)$$

$$A_{contact} = \pi R_d^2 \quad (10)$$

$$t_r = k \gamma^{c A_{contact}} \quad (11)$$

At a constant membrane stress, this model predicts that significant hemolysis only 15 occurs when cell residence time reaches a critical level. If residence time is modulated by pore height, a dramatic increase in hemoglobin is expected beyond a critical pore height, and this is observed in the experimental data. Fig. 25 shows the relationship between pore height squared and hemoglobin level for all pore width test devices, and demonstrates a dramatic increase in hemoglobin with pore height.

The effect of pore geometry on plasma flux may also be evaluated using a residence time model, and the results of this analysis also support the validity of this approach. Pore height was found to be a primary modulator of plasma flux, with decreasing pore height causing a significant increase in flux, while pore width and length did not have a clear and
 5 consistent effect. This result was unexpected, as pore size is not reported to have a significant impact on plasma flux in the plasmapheresis literature.

A residence time model can be used to explain the observed dependence of plasma flux on pore height. Under this hypothesis, plasma flow across the filter occurs between the time a red cell is dislodged from the filter and is replaced by a new cell. Flux is proportional
 10 to the ratio of the time the filter is unoccupied by cells and the total time of operation. This ratio may be modeled using an estimate of the residence time of red cells in the filter and the time required to replug the filter. The time to replug the filter is modeled as an inverse function of shear rate as shown in equation (12), where t_p is the time for a new cell to plug
 the filter, and c_5 and m are constants. The overall equation for plasma flux can then be
 15 developed from equations (11) and (12) resulting in equation (13). Rearrangement and simplification of this equation results in a model for flux as shown in equation (14), where a ,
 b , and k are constants and γ_w is wall shear rate.

A fit of this model to the average flux for all hemolysis test devices is shown in Fig.
 26, and the correlation of the experimental data to the model supports the validity of a
 20 residence time approach.

$$t_p = c_5 \gamma^m \quad (12)$$

$$J_f = k_1 \frac{\text{time pore is open}}{\text{total time}} = k_1 \frac{t_p}{t_p + t_r} = k_1 \frac{c_5 \gamma^m}{c_5 \gamma^m + c_3 \gamma^{c_4 A_{\text{contact}}}} \quad (13)$$

$$J_f = k \gamma_w^{(a+bh_{\text{pore}}^2)^2} \quad (14)$$

The effects of pore height in microfilter plasmapheresis suggest that the physical mechanism of plasma separation and hemolysis are significantly different from membrane plasmapheresis. This difference is likely attributable to two major differences in separation 5 characteristics. First, microfilter membranes are rectangular, and the interaction of the cell with the filter is substantially different than the more circular microporous membrane pores. Secondly, microfilter pores are located at a singular position in the channel, while microporous membrane pores cover the entire area of the flow channel. In a conventional membrane separation operation utilizing flow channels of greater length scale i.e., meter 10 scale, a single trapped red cell in the filter pore is typically surrounded by a localized boundary layer of concentrated red cells. This boundary layer of hydraulically resistive packed cells self-limits the flow of filtrate subfraction. Generally known as "concentration polarization" in the separation science and engineering field that design macro-filtration devices, the hydraulically resistive boundary layer thickness is relatively small compared to 15 flow channel dimensions and thus bulk flow-induced momentum interactions with the boundary layer are somewhat limited. In the microfilter operation, however, blood is freely flowing under the filter pore area, the relative thickness of the boundary layer compared to the microflow channel dimension can be significant. In this smaller scale separation device, trapped red cells at the filter face may have significant interactions with the bulk flow stream 20 that are not possible in conventional membrane separation processes, such as reverse osmosis, ultrafiltration and the microfiltration of complex fluids such as plasmapheresis.

From the foregoing it will be seen then, that the major microfilter design parameters controlling hemolysis in microfluidic-based passive blood separation devices have been identified. Hemolysis is minimized at pore heights < 0.5 μm and filter pore lengths < 100 μm . 25 Surprisingly, plasma flux was substantially increased at shallow pore heights. This was

contrary to the common perception involving membrane filtration. This is a very favorable result for microfilter device as hemolysis is minimized while flux is maximized at small pore heights. The relationship between both flux and hemolysis with filter pore heights may be explained utilizing a residence time model. In this model, cell residence time in the filter is
5 controlled by cell deformation into the filter pore, which is minimized at low pore height.

The controlled lysis of red cells was accomplished by varying the height and length of the filter pore was achieved. This indicates that this technique may be applied to lab-on-a-chip applications where the controlled lysis of target cells is desired. The possibility of the selective lysis of other bloods cell fractions, e.g. white blood cells, from a drop of whole
10 blood is suggested, as well. If such devices could be developed, they would have significant implications in the development of integrated lab-on-a-chip device utilizing miniaturized on-chip Polymerase Chain Reaction (PCR) methods for genomic and proteomic applications as these devices could selectively lyse white blood cells directly from whole blood.

Several diagnostic possibilities present themselves as an outgrow of the above
15 development. The susceptibility of red cells to lysis is a diagnostic technique used in diagnosing certain disease states such as heredity spherocytosis and thalassemia. Diagnostic testing for these conditions is typically performed using a technique known as "osmotic fragility." This test determines the susceptibility of red cell lysis in various concentrations of saline by detecting the release of hemoglobin from lysed red cells. This technique is
20 cumbersome and time consuming. It requires evaluation of a small blood sample in 10 to 20 different concentrations of saline solution which must be individually prepared.

In this example, the determination of red cell fragility is conducted using the passive
microfluidic device described, and the test takes several minutes. A drop of blood is placed
on the inlet reservoir and proceeds to flow through the microchannel for exposure to 5 to 20
25 individual microfilters of various lengths as illustrated in Fig. 7. Each individual microfilter

extracts a small amount of plasma into a plasma outlet channel. The level of stress applied to the blood cells by each microfilter is determined is determined by the length of the microfilter. Longer microfilters apply a greater level of stress. Using a colorimetric technique, the plasma isolated by each microfilter is analyzed for the concentration of hemoglobin. As the level of cell lysis increases the amount of hemoglobin and isolated plasma increases and the degree of red coloration of the isolated plasma can be detected by colorimetric comparison of the isolated plasma to several on-device hemoglobin standards.

A further example of the use of devices of the present invention includes the detection of Sickle cell anemia. sickle cell anemia is a genetic blood disorder that causes anemia and pain. This disease is caused by the presence of defective hemoglobin in the blood cells known as hemoglobin s (Hbs). The presence of Hbs causes the red blood cells to become more rigid and taken on a classic sickle cell shape. "Unlike normal red cells, the sickled red cells cannot squeeze through small blood vessels. They stack up to cause blockages depriving organs and tissues of oxygen-carrying blood. This produces periodic episodes of pain and can lead to damage of tissues and vital organs as well as to other serious medical problems. While normal red blood cells live about 120 days in the bloodstream, sickled red cells die after 20 to 20 days. Because they cannot be replaced fast enough, the blood is chronically short of red blood cells, the condition known as anemia."

Sickle cell diagnosis is typically done using electrophoresis techniques to identify the presence of Hbs. This is a complex technique requiring significant sample preparation, skilled labor and expensive diagnostic equipment. Here, a drop of blood is placed on the inlet to a microfluidic channel comprised of a series of microfilters of varying height from 0.8 μm to perhaps 2 μm . Plasma and red blood cells are isolated in the outlet area of the various filters. Analyzing using colorimetric techniques based on the presence of hemoglobin, sickle cells with reduced deformability and unable to pass across filter dimensions that normal red

blood cells may pass will greatly reduce the degree of red coloration in the filter effluent in sickle cell patients.

Changes in Blood Flow-Deformation Behavior

The present technology can be used to discriminate changes in the flow-deformation behavior of complex fluids such as blood. Using more macroscale devices, known as viscometers or rheometers, Dintenfass previously showed that even small changes in blood-related flow-deformation parameters such as, for example, viscosity's correlation to pre-disease and diseased states.

The present microfluidic devices have the potential capability and fidelity to discriminate small changes in blood flow behavior that may also be useful for the diagnosis of pre-disease, disease and other pathological states expressed via viscosity and related flow-deformation characteristics of complex biological fluids such as blood. One example is, again, Sickle Cell Anemia where the red blood cells take on the characteristic sickle cell shape, the effect of which can be seen in the flow-deformation behavior of blood.

15 Monitoring Capillary Pressure

Shown in Fig. 10, a gauge 250 for monitoring capillary pressure includes an entrapped air bubble 252 encapsulated in a small chamber 253 etched in the Si of a substrate 255. A tiny tube 256 communicates between the chamber 253 and a flow channel 258, like the channels previously discussed. A column 259 of the specimen liquid in the tube 256 acts against the pressure of the entrapped air. The position of the meniscus 260 along the tube 256 is a measure of capillary pressure of the liquid in the channel 258 and tube 256. A constriction 262, of the nature of a filter pore, for example, may be required or efficacious to prevent air escaping the chamber 253 during initial wetting of the channel 258 and the tube 256. Indicia 263 may be etched into the substrate 255 or printed on the glass of a cover plate 25 through which the gauge 250 is viewed.

As shown in Fig. 11, two of the "bubble meters" of the kind illustrated in Fig. 10 can be placed a known distance apart. Similar to a pitot tube, the pressure difference at the two locations is measured. The pressure drop along a long length of flow channel is thus known and from that flow rate of a know liquid can be estimated.

5 Fabrication

Microfluidic devices were fabricated in 100mm diameter silicon and glass wafers. The filter structures and flow channels were etched into silicon wafers, and enclosed by anodically bonding a glass wafer to the silicon wafer. The fabrication process can be broken down into five main areas: photolithography, silicon etching, cleaning, oxidation, and anodic bonding. A summary of the process details for each area is provided below, and a detail step-by-step process is provided in Table 4. A detailed list of the chemicals utilized is provided in Table 5, and equipment detail is provided in Table 6.

Table 4. Step-by-step fabrication detail for microfilter devices.

Process	Parameters	Time	Equipment	Comments/Notes
Pattern Filter Area				Starting material: 100mm silicon wafers
Dehydration Bake	95° C	2 min	Oven	
HMDS Application	4000 RPM	30 sec	Spinner	
Resist Application	4000 RPM	30 sec	Spinner	Dispense 1-2 ml resist, ~ 1µm thick
Soft Bake	80°C	15 min	Oven	
Exposure	Contact print	15 sec	Canon	Test exposure and adjust as required
Develop in Beaker	Developer	~3 min	Wet Bench	Over develop by 50%
DI Rinse			Sprayer	
N ₂ Dry			N ₂ Gun	
Hard Bake	95°C	2 min	Oven	Dry wafer
Measurement		Dektak		Pre-measurements for etch
Filter Etch				
Plasma Etch SF ₆	See Table A.5		Plasma Lab	Adjust etch time to meet target
Measurement		Dektak		Verify etch depth meets target
Clean				
Strip Resist	Microstrip	~5min	Hot plate	Heat stripper ~40-50C, use agitation
Sulfuric Clean	95°C	10 min	Wet Bench	Spike sulfuric tank with ~200ml peroxide
Rinse/Dry			QDR/N ₂	4 cycle quick dump rinse cycle, dry with N ₂
Pattern Channels				
Dehydration Bake	95°C	2 min	Oven	
HMDS Application	4000 RPM	30 sec	Spinner	
Resist Application	4000 RPM	30 sec	Spinner	Dispense 1-2 ml, ~ 1µm thick
Soft Bake	80°C	15 min	Oven	
Exposure	Contact print	15 sec	Canon	Align mask to filter pattern
Develop in Beaker	Developer	~3 min	Wet Bench	Over develop by 50%
DI Rinse		~30sec	Sprayer	
N ₂ Dry			N ₂ Gun	
Hard Bake	95°C	2 min	Oven	
Measurement		Dektak		Pre-measurements for etch

Etch Channels

Plasma Etch SF₆ See Table A.5 Plasma Lab Adjust etch time to meet target

Measurement Dektak Verify etch depth meets target

Clean

Strip Resist	Microstrip	~5min	Hot Plate	Heat stripper ~40-50C, use agitation
Sulfuric Clean	95°C	10 min	Wet Bench	Spike sulfuric tank with ~200ml peroxide
Rinse/Dry			QDR/N ₂	4 cycle quick dump rinse cycle, dry with N ₂

Measurement Dektak Measure main channel and filter depth

Wet Oxidation

Wet Oxidation	1050°C	~80min	Furnace	Steam oxidation, target ~2100Å
Measure Thickness			NanoSpec	Measure oxide thickness

Prepare Glass

Drill Access Holes Glass Wafers Dremel Dremel drill press, and diamond tipped bits

Clean Wafers

Organic Clean	Microstrip	~5min	Hot plate	Heat until warm to touch, agitate frequently
Sulfuric Clean	95°C	60 min	Wet Bench	Spike sulfuric tank with ~200ml peroxide
Rinse/Dry			QDR/N ₂	

Anodic Bonding

Align Wafers				Visually align wafers by hand
Bonding	~550°C, ~2000VDC	15min	Hot Plate/DC Power Supply	Process until wafers are completely bonded

Table 5. Typical chemicals and materials used in microdevice fabrication.

Chemical	Trade Name	Purpose	Supplier
Sulfuric Acid	Sulfuric Acid Semi Grade	Cleaning	Ashland Chemical (Columbus, OH)
Hydrogen Peroxide	Hydrogen Peroxide Semi Grade	Mix with sulfuric acid for cleaning	Ashland Chemical (Columbus, OH)
Photoresist	OCG 825 27CS	Photolithography	Arch Chemical (Norwalk, CN)
Photoresist Developer	OCG 945 developer	Development of exposed photoresist during photolithography	Arch Chemical (Norwalk, CN)
Organic Resist Stripper	Microstrip 2001	Removal of exposed photoresist	Arch Chemical (Norwalk, CN)
100 mm Glass Wafers, 1.1 mm Thick	Borofloat	Device substrate	Technical Glass Products (Painesville Twp., OH)
100 mm Silicon Wafers, 0.5mm Thick	Silicon	Device substrate	Wacker (Portland, OR) both <100> and <111> wafers with various doping levels have been used successfully
Hexamethyldisilizane	Adhesion Promoter	Resist adhesion	Arch Chemical (Norwalk, CN)

Table 6. Equipment detail for microdevice fabrication.

Equipment	Manufacturer	Model	Purpose
Wet Bench	Custom		Cleaning, photoresist development, and removal
Heated, Quartz Lined Chemical Tank	PCT Systems (Fremont, CA)	90	Controlled heating of sulfuric/hydrogen peroxide solutions for cleaning
Resist Spinner	Specialty Coating Systems (Indianapolis, IN)	P6204	Resist coating
Bake Oven	Blue M (Williamsport, PA)		Resist baking
Photolithographic Contact Aligner	Canon (Lake Success, NY)	PLA-501F	Exposure of photoresist using photomask
Photolithographic Contact Aligner	Suss Microtec (Waterbury Center, VT)	MJB 3	Exposure of photoresist using photomask
Reactive Ion Etcher	Oxford Instruments (Concord, MA).	Plasma Lab Micro RIE 80	Silicon etching
Hot Plate	Various		Heating of organic resist stripper, anodic bonding
Spin-Rinse-Drier	Verteq (Santa Ana, CA)	1600-44	Rinsing of wafers after cleaning
Surface Profiler	Sloan Technology /Veeco Instruments (Woodbury, NY)	Dektak IIA	Measurement of device channel heights and widths
Film Thickness Analyzer	Nanometrics (Milpitas, CA)	NanoSpec AFT	Measurement of resist thickness and silicon oxide layers
Oxidation Furnace	Amtech Systems (Tempe, AZ)	Tempress 8 Stack	Growth of thermal oxide on silicon
High Voltage DC Power Supply	LKB Instruments (Rockville, MD)	LKB 2103	Anodic Bonding
Surface Thermometer	PTC Instruments (Los Angeles, CA)	573C	Monitoring temperature during anodic bonding

Photolithography

Photolithography was utilized to etch the filter pore and flow channel patterns into the silicon wafer using reactive ion etching. Two masking layers or masks 203 (Fig. 5) are required to define a microfilter, the shallower first layer patterns the filter pore structures, and 5 the second masking layer, aligned to the first pattern, defines the flow channels. Positive photoresist was utilized with a dark field photomask to perform these operations.

The photolithographic process starts by baking the wafer in a convection oven for several minutes at 95°C to remove moisture. After the initial bake, hexamethydisilazane (HMDS) is applied to improve resist adhesion. About 0.5ml of HMDS is applied to the 10 silicon wafer, and the wafer is spun using a spin coater at ~4000 RPM for 30 sec to spread the HMDS across the wafer. After HMDS application, ~1-2ml of photoresist 204 is dispensed on the center of the wafer. Again using the spin coater, the wafer is spun at 4000 RPMs for 30 seconds to spread the resist evenly across the wafer. After spin coating, the wafers are baked in a convection oven (soft bake) at 80° C for 15 minutes to remove solvents from the resist 15 and improve mechanical stability of the film. Soft bake time and temperature are important as these factors affect the photospeed of the resist, i.e. high temperatures will destroy the photoactive components of the resist.

Using a photomask 203, selective areas of the resist-coated wafer are exposed with ultraviolet light (UV) in a contact aligner, thereby transferring the mask pattern to the 20 photoresist. The UV exposed photoresist is selectively removed as shown at 205 by immersing the wafer in a beaker of photoresist developer solution. The wafer is gently agitated during development, and the development time is determined by a visual assessment of the wafer. After development, the wafers are rinsed with DI water, and dried using a nitrogen (N₂) gun. Wafers are then baked in a convection oven to ensure all water is removed,

remove solvents, and improve mechanical stability of the film (hard bake). After the hard bake, the wafer is ready for etching or other selective manipulation of the wafer surface.

Silicon etching

Lithographic patterns are transferred to the silicon wafer using reactive ion etching
 5 with sulfur hexafluoride (SF_6) as indicated at 207. Prior to the silicon etch step, a brief silicon oxide etch is performed using a CF_4 and oxygen , O_2 , plasma to help remove native oxide on the silicon surface. The main silicon etch is then performed using SF_6 . Typical etch conditions are provided in Table 7.

Table 7. Typical silicon plasma etch process

Step	Purpose	Gas (sccm)	Power (watts)	Pressure (mTorr)	Time (min)	Notes
1	Pump Down	None	0	0	Wait	Pump down to minimum pressure.
2	Oxide Etch	3 O_2 3 CF_4	120	75	2	This brief oxide etch intended to remove native oxide. Wait for gas and pressure to stabilize prior to turning on RF.
3	Pump Down	none	0	0	Wait	Wait for minimum pressure.
4	Silicon Etch	25 SF_6	120	20	Various	Etch time adjusted to meet experimental targets. Wait for gas and pressure to stabilize prior to turning on RF. Etch rate ~0.8um/min on N-type <100> silicon
5	Pump Down	None	0	0	Wait	Pump out chamber prior to venting.
6	Vent	N_2			Wait	Automatic equipment cycle, pumps down chamber and then vents to atmosphere with nitrogen.

Wafer cleaning

Cleaning is a critical step in device fabrication. Debris or other contaminants left on
 10 the wafer can act as an etch mask and ruin the lithographic pattern. Additionally, particulates and organic contamination can prevent uniform bonding of silicon to the glass. In this

fabrication process, cleaning was accomplished using a commercial organic stripper (Microstrip 2001) and a hot solution of sulfuric acid and hydrogen peroxide (piranha).

The organic stripper is used to remove photoresist and organic contaminates. Wafers are immersed in a beaker of the warm stripper, ~40-50°C, and gently agitated. Agitation of
5 the solution greatly improves cleaning, and helps to reduce process time. When cleaning resist from wafers, visible resist debris will appear in the cleaning solution. Wafers should not be removed from the organic stripper until all resist debris is broken down, or this material will contaminated the wafer when it is removed from solution. After cleaning with the organic stripper, wafers were cleaned in a mixture of hot sulfuric acid and hydrogen peroxide,
10 ~95°C. This solution is maintained in a temperature-controlled tank by CSER personnel. Approximately 200ml of hydrogen peroxide was typically added to the sulfuric tank immediately prior to cleaning wafers.

Rinsing and drying of the wafers after cleaning is critical, a thorough rinsing with DI water helps to remove particulates. Particulates allowed to dry to a wafer are very difficult to
15 remove. After cleaning in hot sulfuric, wafers were rinsed in quick-dump rinser (QDR) for four cycles, and then dried using a nitrogen gun.

The photolithography steps are performed twice; once for the shallower filter pores and once for the deeper flow channels.

Oxidation

20 Wafers were oxidized to improve the visibility of liquids in the microfluidic channels. As illustrated in Fig. 12, thin oxide layer 215, ~2100A, on the silicon surface alters an optical interference effect that changes the color of the wafer. When a liquid wets this surface, the critical angle of reflection is changed, and the observed color of the surface changes visibly.

This greatly improves visualization of fluid flow via capillary action in very shallow channels. Wafers were oxidized using an oxidation furnace in steam at 1050°C.

Light from a source 217 impinges the oxide layer 215. Depending on the angle of incidence, the light will be partially reflected and the refracted portion will emerge after 5 reflection from the interface 218 between the silicon and silicon dioxide layer to interact with the reflected portion of light. A clear specimen 216 alters the critical angle and, depending on the angle at which the illuminating source 217 is supported can either eliminate the interference effect that altered the appearance of the silicon dioxide layer 215 or create an interference pattern if one was not present previously. The change effected by the clear 10 specimen 216 in the interference perceived by an observer 222 makes the presence of the specimen 216 apparent.

Wafer bonding and glass preparation

Glass wafers 201 are anodically bonded as closures to the patterned silicon wafers. Prior to bonding, multiple access holes (such as the holes 45, 52 and 55 of Fig. 1), ~1mm 15 diameter, are drilled through the glass wafer using diamond tipped drill bits (similar to dental drill bits), a Dremel tool, and Dremel drill press. Prior to drilling, the glass wafer is adhered to a metal substrate with double-sided foam tape. This helps to dampen vibration in the glass and prevent fracture. The foam tape also provides a cushion to the wafer, and minimizes stress points that occur due to particulates on the glass. The mounted wafer is submersed in a 20 pan of shallow water to cool the bit during drilling. Glass wafers are very susceptible to breakage, and drilling must be done slowly with minimal applied pressure. Drilling is done using a pulsed action of the drill bit. The pulsed action prevents excessive heating of the bit, which improves bit lifetime. The diamond coating is degraded by heat, and lifetime of a bit is marginal, ~50 holes. After drilling the wafer is released from the foam tape by soaking in

isopropyl alcohol. This procedure has been used to successfully drill ~75 holes in a single glass wafer.

Prior to bonding the glass must be cleaned thoroughly to remove particulates and other contaminates. Cleaning is performed using the organic stripper and hot 5 sulfuric/peroxide as previously described. After cleaning, the silicon and glass wafers are aligned by hand and pressed together at a single contact point to establish a preliminary bond. A small amount of room temperature contact bonding will occur if the two surfaces are clean and flat. If a preliminary bond cannot be established, or if significant grinding sounds are heard when the wafers are contacted, the wafer pair is unlikely to bond using anodic bonding. 10 Wafers should be re-cleaned as necessary until a preliminary room temperature bond can be achieved.

After cleaning and preliminary bonding, the wafer is transferred to a hot plate, 275, for anodic bonding, in known fashion, as shown in Fig. 14. Anodic bonding occurs due to 15 electrically induced movement of sodium ions in the glass away from the glass/silicon interface. A positive voltage is applied by an electrode 278 to the silicon wafer 200 and a negative voltage or ground is applied to the glass wafer 201 by an electrode 280. The silicon wafer is biased by placing it on the metal electrode 278 isolated from the hot plate surface using a glass plate 286. The glass wafer is biased using a heavy copper grounding rod with a pointed tip as the upper electrode 280. The wafer is heated to ~550°C and up to 2000V is 20 applied across the electrode 278, 280. Higher temperature and voltages increases the mobility of sodium ions and speed the bonding process, however bonding may be achieved at lower temperatures and voltage levels. The bonding process typically starts at a single contact point on the two wafers and spreads across the whole surface over time. Some un-bonded areas should be expected on every wafer-pair due to the presence of particulates or 25 contamination. The quality of the cleaning technique used on the silicon wafers throughout

the fabrication process has a large impact on the number of bonding defects present in the final devices.

Comparison with Membrane Plasmapheresis

Passive microfilter device operation has similarities with microporous membrane plasmapheresis. For example, similar to microporous membrane, microfilter plasma flux is dependent upon wall shear rate raised to a fractional power and over the experimental range, the shear rate exponent varies between 0.55 and 0.76. This falls well within the range of exponent values reported for microporous membrane plasmapheresis. To compare the relative flux levels achieved in microfilter device tests to macroscale plasmapheresis performance, the model established by the experimental data obtained by the inventors was compared to the microporous membrane filtration model of Zydny and Colton, equation as shown in Fig. 27. On the basis of the model predictions, significantly greater microfilter plasma flux levels can be achieved (on the order of 2-5x higher) than can be achieved with microporous membrane systems. The surprisingly higher level of flux achieved in microfilter systems may be attributable to the significant physical differences between the two systems. Equations 15 and 16 represent the generalized and specific device plasma transport/flux performance heuristic that leads to the unexpected result shown in Fig. 27.

$$J_f = k \gamma_w^{(a+bH_{pore}^2)^2} \quad (15)$$

$$J_f = 0.62 \gamma_w^{(0.885-0.166H_{pore}^2)^2} \quad (16)$$

The key difference between these two systems may be due to the filter pore distribution which is hypothesized as the primary factor responsible for the higher level of plasma flux in the microfilter system over the membrane process. In microporous membrane processes, a continuous fouling layer is formed over the entire surface of the blood-filter interface, and the interaction of the membrane with the bulk channel flow is limited to the

outer surface. However, in weir style microdevices, the singular location of the filter pores at the top corner of the flow channel allows for greater interaction between the filtration channel blood flow and the fouling layer. Also, the filtration channel blood flow in microfilter devices surrounds the boundary layer on two sides, i.e. at the outer edge and underneath the fouling 5 layer. This additional interaction with the surrounding bulk flow may increase plasma flux by reducing the fouling effect of the cells through decreased red blood cell residence time in the filter pore, or possibly an increased shear enhanced diffusion of red cells resulting in a decreased in boundary layer thickness, or both.

In membrane plasmapheresis, the effect of hematocrit on flux is well noted in the 10 literature. The theoretical models decrease in plasma flux with increasing hematocrit. However, as mentioned, microfilter plasma flux was not significantly modulated by hematocrit between 19 to 40%. While the reason for this difference is unknown and warrants further characterization, this surprising insensitivity is inherently advantageous to clinical applications where significant sample-to-sample variations in hematocrit are present.

15 A Generalized Device Operational Model

Device modeling was performed in two stages. First, the blood flow through the device was modeled to estimate both the average wall shear rate in the filtration channel, as well as, the time of operation. Secondly, plasma flux was calculated based on the average 20 wall shear rate. Using this result, the total volume of plasma extracted would then be calculated based on flux, microfilter dimensions, and the time of operation.

In the operational model, blood flow through the devices was predicted with classical hydrodynamic models assuming constant apparent viscosities for blood in the main filtration channel and the expanded channel. This methodology has limited utility, but in the absence of more comprehensive flow models that accurately predict the complex behavior of capillarity-

driven blood flow, this method enables basic microdevice blood flow predictions, which are requisite to overall operational model development.

An assumed blood viscosity of 0.003 Pa-s in the main flow channel and an assumed blood viscosity of 0.040Pa-s in the expanded channel were based on the experimental observations. Both of these assumed viscosities are consistent with blood having nominal hematocrit values in the range of ~35-45%. Device modeling for blood with hematocrit levels outside this ranges will require adjustment to these assumed apparent blood viscosity parameters.

In the overall microfiltration operational model, blood flow through the expanded channel section of the microfilter devices was modeled using the device layout and parameters shown in Fig. 28. In the hydrodynamic model, the total pressure drop over the device was assumed to be constant, and it was estimated from the apparent capillary pressure in the expanded channel. Using the viscosity assumptions stated above, the average velocities in the expanded and filtration channels were calculated in an iterative manner until the relationships shown in equations (17 - 20) were satisfied. These relationships are based on a constant total pressure drop, and satisfying continuity requirements.

$$Q_n = Q_e \quad (17)$$

$$P_{cap} = \Delta P_n + \Delta P_e \quad (18)$$

$$\Delta P_e = \frac{3u_e L_e \mu_e}{k_{e2-d} h^2} \quad (19)$$

$$\Delta P_n = \frac{3u_n L_n \mu_n}{k_{n2-d} h^2} \quad (20)$$

Once the expanded channel velocity was calculated using the flow model described above, it was then used to predict the advancement of blood in the expanded channel at one-second time intervals per equation (21) to determine values of L_e over time. Using the new

value of L_e , the expanded and narrow filtration velocities are again calculated in an iterative manner as described above. The model calculations proceed in this iterative fashion until the wall shear rate in the expanded channel reaches the minimum level of $<75 \text{ s}^{-1}$. This design constraint determines the end-point for flow in the expanded channel, thus defining the total time of operation and total length of the expanded channel. The overall result of this quasi steady state flow model is an estimate of velocity in the expanded and filtration channels over the operating time of the device.

$$L_e(t+1) = L_e(t) + V_e(t) \quad (21)$$

Once the blood velocity in the expanded channel is determined, it is used to calculate the time-averaged wall shear rate in the filtration channel. Plasma flux is then predicted using the empirical wall shear rate model previously discussed in Chapter 6, equation (16). Total plasma volume extracted can then be calculated based on the plasma flux, time of operation, and filter area. Additionally, the average change in blood hematocrit due to fractionation of plasma was estimated. This result is used to verify compliance to the design rule specifying a maximum allowable change in hematocrit of $\leq 0\%$.

As part of the model development and validation, the experimental results obtained from Weir 11 devices, which met the design rule for maximum allowable change in hematocrit, were compared to the operational model predictions. Figure 29 shows the model results for plasma volume fractionated versus the experimentally observed values. As can be seen, the model predictions are in good agreement with the experimental results.

The operational model can be utilized to calculate microfilter device performance over a wide range of device dimensions, which can be used to determine optimum designs. The key device dimensions include filtration channel length, channel height, and total width of the expanded channel. An example of the operational model output is shown in the response surface plot of Figure 30. This plot shows the predicted relationship between total

fractionated plasma volume, filtration channel length and expanded channel width. Using the resulting color model to visualize and compare various combinations of design parameters indicates that there are combinations of expanded channel dimensions and filtration channel lengths where optimum device performance is attainable. In this case, the maximum volume
5 of plasma fractionated determines the optimum performance criteria.

When the operational model is applied over a wide range of device dimensions, a portion of the evaluated design space typically will not satisfy the design rule requirements for the minimum expanded channel wall shear rate or maximum change in hematocrit or both. If these constraints are not met, the operation model assigns a zero value for the plasma
10 volume isolated. For example, the upper right corner of Fig. 30 shows the portions of the design space that did not meet the minimum shear rate requirement, and this area was assigned a zero plasma volume value. In a second example of operational modeling (Fig.
31A&B), the effect of both the hematocrit and minimum wall shear rate design rules on the usable design space can be seen. The top panel of Fig. 31A&B shows an operational model
15 that is unconstrained by the hematocrit design rule, and the bottom panels shows the reduction in available design space when the hematocrit design rule is applied. The hematocrit design rule significantly reduces the usable design space, and prohibits the attainment of the optimal design condition. Recall that the hematocrit design rule was implemented to avoid a large increase in apparent expanded channel viscosity and associated
20 blood instability. In part this constraint is due to the formation of a blood cell plug at the leading edge of the flow, and this example, once again, illustrates how this phenomenon negatively impacts device design and optimization.

Although preferred embodiments of the invention have been described in detail, it will be readily appreciated by those skilled in the art that further modifications, alterations

and additions to the invention embodiments disclosed may be made without departure from the spirit and scope of the invention as set forth in the appended claims.

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We claim:

1 1. A method of making an implement for use with treatment or analysis of a
2 liquid, comprising:

3 (a) devising a set of heuristic rules from the behavior of the liquid on a
4 scale of the scale of the instrument to be made,

5 (b) fabricating as a part of the instrument a liquid contacting device based
6 upon at least one of the heuristic rules, and

7 (c) providing a means of determination of a characteristic of the liquid
8 based on the liquids behavior in contact with the liquid contacting device.

1 2. An instrument for the observation, treatment or analysis of a liquid made by
2 the method of claim 1.

1 3. The method according to claim 1, wherein the liquid comprises a suspension
2 of cells, devising the heuristic rules including determining parameters of the liquid contact
3 device effecting lysis of the cells, and implementing selected values of the parameters to
4 provide the desired presence or absence of lysis of cells.

1 4. The method according to claim 3, wherein the liquid is blood, the cells are
2 blood cells suspended in plasma, the parameters include cell stress and cell stress duration.

1 5. The method according to claim 4, wherein the blood cells are red blood cells.

1 6. The method according to claim 5, wherein cell stress is determined as a
2 function of the size of a filtration opening that is sized to prevent red blood cell passage and
3 cell stress duration is determined as a function of length of a plasma path along a passage
4 leading plasma from the filtration opening.

1 7. The instrument according to claim 2, wherein the instrument comprises a
2 liquid input opening, a first passage leading from the liquid input opening and operative to
3 move liquid therealong by capillary action, a filter communicating between the first passage
4 and a path of liquid filtrate flow operative to move liquid filtrate therealong by capillary
5 action, the first passage leading to an expanded liquid flow path for drawing liquid therealong
6 by capillary action.

1 8. The instrument according to claim 7, wherein the expanded liquid flow path
2 comprises a plurality of liquid flow paths connected to receive liquid flow in parallel from the
3 first passage and of a size to move the liquid filtrate therein by capillary action.

1 9. The instrument according to claim 7, wherein the path of liquid filtrate flow
2 leads to an output location for the liquid filtrate.

1 10. The instrument according to claim 7, wherein the path of liquid filtrate flow
2 leads to a testing location having analytical provisions associated therewith for analysis of the
3 filtrate.

1 11. The instrument according to claim 10, wherein the analytical provisions
2 include electrooptical means for illuminating the filtrate liquid and for receiving light from
3 the illuminated filtrate liquid.

1 12. The instrument according to claim 11, wherein the electro-optical means
2 comprises a laser, a first reflective sidewall at the testing location positioned to direct laser
3 light from the laser through the test location, a photodetector, and a second reflective sidewall
4 at the testing location positioned to direct laser light from the test location to the
5 photodetector.

1 13. A blood separation instrument comprising an input opening to an input
2 location for receiving whole blood, a first blood flow channel communicating with the input
3 opening and being of a size to cause blood flow therein from the input location by capillary
4 action, a filter opening into a side of the blood flow channel, the filter having at least one
5 opening therethrough smaller than a red blood cell, a blood plasma collection location for
6 receiving plasma from the filter, an expanded blood flow channel in communication with the
7 first blood flow channel and having defined therein a plurality of parallel connected channels
8 sized to draw blood therethrough by capillary action.

1 14. The blood separation instrument according to claim 13, wherein the filter
2 comprises a weir formed at the side of the blood flow channel, the weir constricting a slit-like
3 opening through the side of the blood flow channel to a height less than the height of the
4 blood flow channel.

1 15. The blood separation instrument according to claim 13, further comprising a
2 blood plasma flow channel in communication with the slit and of a size to draw plasma
3 through the slit by capillary action.

16. The blood separation instrument according to claim 15, wherein the length of
the plasma flow channel is sufficiently short as to shorten time of plasma flow therein below
a duration such as ordinarily causes lysis of red blood cells adhered to a slit-like opening the
size of the slit-like opening through the side of the blood flow channel.

1 17. An instrument for monitoring capillary pressure including:

- (a) an entrapped gas encapsulation,
 - (b) a path of liquid flow of a cross-section that causes capillary flow of the liquid therein,
 - (c) a tube in communication between the encapsulation and the tube having a diameter such that, under capillary pressure of a path of liquid flow, capillary pressure in the liquid is indicated by a in the tube acting against and compressing the gas in the encapsulation, the tube being sufficiently transparent or translucent as to allow the liquid therein to be detected.

18. The instrument according to claim 17, wherein the entrapped gas is air.

1 19. The instrument according to claim 17, wherein a cross-sectional dimension of
2 the path is about 20 μm or less.

1 20. The instrument according to claim 17, further comprising a filter pore
2 constricting an input opening from the path into the tube.

1 21. A liquid flow meter including the instrument of claim 17 and having a further
2 instrument for monitoring capillary pressure spaced along the path of liquid flow.

1 22. A method of illuminating a substantially clear liquid specimen for observation
2 comprising:

- 3 (a) providing a substrate,
- 4 (b) providing an at least partially light transmitting layer on the substrate
5 to form a specimen support surface and having an interface with the substrate,
- 6 (c) placing the liquid specimen on the specimen support surface, and
- 7 (d) illuminating the specimen by:
 - 8 (i) directing light onto the specimen support surface at an angle
9 selected to cause partial reflection at the specimen support surface to divide
10 illuminating light into refracted light and reflected light,
 - 11 (ii) reflecting the refracted light from the substrate at the interface
12 of the layer and the substrate and through the layer to cause visible interference with reflected
13 light that is reflected from the specimen support surface.

1 23. The method according to claim 22, wherein step (b) comprises providing an
2 oxide layer on the substrate.

1 24. The method according to claim 22, wherein step (a) comprises providing a Si,
2 substrate, and step (b) comprises providing a SiO₂ layer on the Si substrate.

1 25. The method according to claim 22, wherein step (b) comprises providing a
2 thin layer in light interference effecting relation to the substrate.

1 26. A liquid specimen handling device, including:

- 2 (a) a substrate,
- 3 (b) a layer of at least partially light-transmitting material on the substrate
4 and forming an interface therewith and having an upper specimen support surface, and
- 5 (c) illumination means mounted to direct light into the specimen at an
6 angle causing partial reflection at the specimen support surface, to divide illuminating light
7 into refracted light and reflected light, and to cause interfering intersection of the refracted
8 light reflected from the substrate-layer interface and the reflected light from the specimen-
9 support surface.

1 27. The device according to claim 26, wherein the layer is an oxide of the material
2 of the substrate.

1 28. The device according to claim 27, wherein the substrate is Si and the layer is
2 SiO₂.

1 29. A liquid specimen test device comprising:
2 (a) a plurality of liquid flow channels defined in a substrate,
3 (b) a plurality of filter openings communicating between the liquid flow
4 channels and a plurality of filtrate collection regions,
5 (c) at least one liquid input reservoir connected in liquid communication
6 with
7 the flow channels,
8 (d) a plurality of expanded output flow channels downstream of the liquid
9 flow channels,
10 (e) a closure covering the flow channels, the filters, the collection regions
11 and
12 the expanded output flow channels, and
13 (f) at least one vent line connecting the collection regions and the
14 expanded
15 flow channels to at least one opening to atmosphere.

1 30. The device according to claim 29, further comprising at least one liquid input
2 opening through the closure to the liquid input reservoir for the input of a liquid test
3 specimen.

1 31. The device according to claim 29, wherein the expanded output flow channels
2 contain multiple capillary flow paths for drawing liquid of a specimen along the paths by
3 capillary action.

1 32. The device according to claim 29, wherein the filter openings comprise weir-
2 type filters opening into the liquid flow channels.

1 33. The device according to claim 32, wherein the liquid flow channels have
2 plural weir-style filters opening thereto and leading to separate filtrate collection regions.

1 34. The device according to claim 33, wherein the substrate is a semiconductor
2 substrate and the closure comprises a glass lid secured thereto.

1 35. The device according to claim 35, wherein the flow channels comprise at least
2 eight flow channels, each of the channels having a filter opening thereto and each flow
3 channel leading to one of at least eight expanded output flow regions.

1 36. The device according to claim 31, wherein the capillary flow paths in the
2 expanded output flow channels are connected in parallel to an associated flow channel.

1 37. The device according to claim 36, wherein the parallel capillary flow paths are
2 serpentine.

1 38. The device according to claim 36, wherein the substrate is a semiconductor
2 chip formed from a single semiconductor crystal wafer.

1 39. The device according to claim 38, wherein the semiconductor crystal is Si.

1 40. The device according to claim 39, in which the liquid flow channel cross-
2 sectional dimensions are sized to effect movement of liquid of a liquid specimen therein by
3 capillary action.

1 41. The device according to claim 40, wherein the liquid flow channel has a cross-
2 sectional dimension a, where:

3 $0.3 > a > 0.1 \mu\text{m}$.

1 42. The device according to claim 41, wherein the cross-sectional dimension a is
2 about $0.5 \mu\text{m}$.

1 43. A method of cell lysis comprising:

2 (a) moving a liquid suspension of cells in a flow path by capillary action,
3 (b) providing a filter opening into the flow path, the filter having one or
4 more pores sized to engage and retain cells in the suspension and having a length in the
5 direction of liquid flow through the filter sufficiently long to cause lysis of at least some
6 retained cells in the suspension as a function of the stress on the cell and the duration of its
7 retention at the filter pore.

1 44. The method according to claim 43, wherein step (a) comprises moving blood
2 in the flow path, and step (b) comprises providing a filter having one or more pores of a size
3 and length sufficient to cause lysis of at least a portion of the red blood cells in the blood.

1 45. The method according to claim 43, further comprising moving lysed cells and
2 liquid out of the filter through a channel by capillary action to a collection region.

1 46. The method according to claim 44, further comprising moving the lysed red
2 blood cells and plasma from the filter along a channel by capillary action to a collection
3 region.

1 47. The method according to claim 43, wherein step (a) further comprises moving
2 the liquid suspension past the filter to an expanded channel having multiple paths for moving
3 the liquid suspension therein by capillary action.

1 48. The method according to claim 43, wherein step (b) further comprises
2 providing a plurality of filters of varying filter pore geometries along the length of the flow
3 path to effect higher and lower rates of lysis at the filters.

1 49. The method according to claim 48, further comprising using the lysis observed
2 as an indicator of Sickle Cell Anemia.

1 50. The method according to claim 48, wherein providing a plurality of filters
2 comprises providing a plurality of filters of differing pore lengths.

1 51. The method according to claim 48, wherein providing a plurality of filters
2 comprises providing a plurality of filters of differing pore widths.

1 52. The method according to claim 48, wherein providing a plurality of filters
2 comprises providing a plurality of filters of varying pore heights.

1 53. A method of fabricating a passive, liquid specimen handing device including:

2 (a) providing a semiconductor substrate;

3 (b) applying a photoresist to the substrate;

4 (c) providing a mask defining liquid flow channels of cross-sectional

5 dimensions suitable to induce capillary action flowing of the liquid therein;

6 (d) exposing the photoresist to U.V. light through the mask;

7 (e) removing the photoresist in locations exposed to light through the

8 mask;

9 (f) etching the semiconductor in areas revealed by removing the

10 photoresist to

11 form the liquid flow channels and other features of the device; and

12 (g) securing a closure layer to the semiconductor substrate over the etched

13 channels and other features of the device.

1 54. The method according to claim 53, wherein the closure layer is glass and step

2 (g) comprises anodic bonding the glass closure layer to the semiconductor substrate.

1 55. The method according to claim 54, wherein the semiconductor is Si.

1 56. The method according to claim 53, wherein step (c) comprises providing a

2 mask defining a liquid flow channel sized to induce capillary action in the liquid of an

3 intended specimen type and having an expanded downstream channel section in

4 communication with the liquid flow channel and with a plurality of parallel connected liquid

5 flow paths therein each of a cross-sectional dimension to induce parallel capillary action flow

6 of the liquid.

1 57. The method according to claims 56, further comprising separately from

2 steps (c), (d), (e) and (f), conducting the steps of applying a photoresist, providing a mask

3 defining filter pores to be etched into the semiconductor surface, exposing the photoresist to

4 U.V. light through the mask, removing the photoresist in filter pore areas exposed through the

5 mask, and etching the pore areas of the semiconductor substrate to a lesser depth than a

6 preselected depth of the flow channels.

- 1 58. A micro-engineered blood separation device including:
- 2 (a) a substrate,
- 3 (b) a cover plate,
- 4 (c) a blood inlet reservoir,
- 5 (d) a blood outlet reservoir,
- 6 (e) a blood flow channel etched into the substrate and connecting the
- 7 blood inlet reservoir and the blood outlet reservoir
- 8 (f) an area of microfilter etched into the surface, pores of the microfilter
- 9 having an opening into the blood flow channel, the pores having a cross-sectional dimension
- 10 less than 10 μm ,
- 11 (g) a plasma outlet channel etched in the surface of the substrate in
- 12 communication with the pores at ends thereof opposite the ends opening into the blood flow
- 13 channel, and
- 14 (h) a plasma outlet reservoir connected with the plasma outlet channel.

- 1 59. A blood separation instrument comprising:
- 2 (a) a blood inlet opening;
- 3 (b) a first reservoir connected with the blood inlet opening;
- 4 (c) a blood outlet opening;
- 5 (d) a second reservoir connected with the blood outlet opening;
- 6 (e) a blood flow path from the first to the second reservoir;
- 7 (f) a plurality of micro-channel blood filters communicating with the
- 8 channel between the first and second reservoirs;
- 9 (g) each of the micro-channel blood filters comprising:
- 10 (i) a plurality of micro-channels having at least one cross-sectional
- 11 dimension less than 1.0 μm in communication with the channel; and
- 12 (h) the length of the micro-channel of each filter differing in length from
- 13 the length of the micro-channels of each other filter.

1 60. A method of measuring % hematocrit of a blood specimen including:

- 2 (a) providing a substrate having a blood flow channel thereon leading
3 away from an input region and having a serpentine path to a vented location,
4 (b) the blood flow channel being of a cross-dimensional size to effect flow
5 of the blood sample by capillary action therein,
6 (c) introducing a blood specimen to the input region; and
7 (d) determining how far along the blood flow channel the blood from the
8 specimen flows by capillary action as a function of % hematocrit.

1 61. A % hematocrit testing device for use with a blood specimen including:

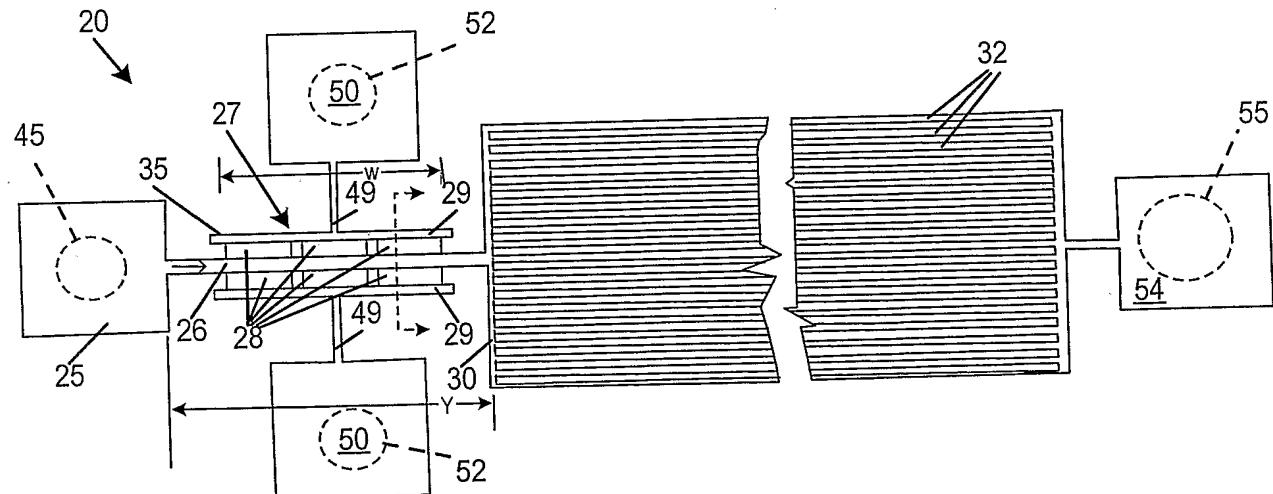
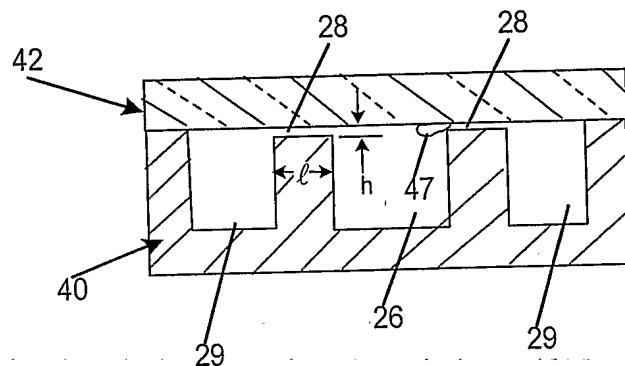
- 2 (a) a substrate;
3 (b) an input region,
4 (c) a blood flow channel formed in the substrate and communicating with
5 the input region,
6 (d) the blood flow channel being of a cross-sectional dimension that will
7 effect blood flow by capillary action,
8 (e) a vent opening to the blood flow channel remote from the input region,
9 whereby the distance along the blood flow channel that blood from a specimen travels from
10 the input region is a function of the % hematocrit of the blood of the specimen.

1 62. The hematocrit testing device of claim 61, wherein a portion of the blood flow
2 channel intermediate the input region and the vent is of serpentine configuration.

1 63. The hematocrit testing device of claim 61, further comprising flow-slowing
2 chambers formed in the substrate in the path of blood flow in the blood flow channel.

1 64. A method of designing a device including:

- 2 (a) operational modeling the device by applying known relationships of
3 theoretical operations features of the device to define a design space,
4 (b) fashioning the actual operational features of the device from within the
5 constrained design space.

**Fig. 1****Fig. 2**

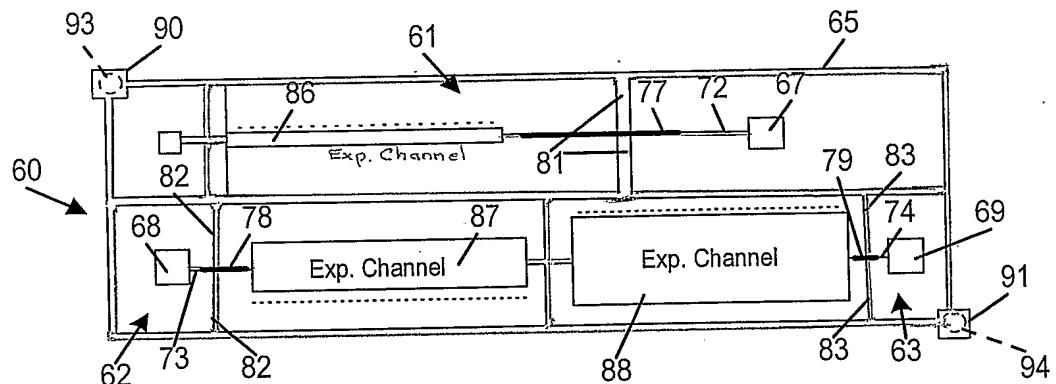


Fig. 3

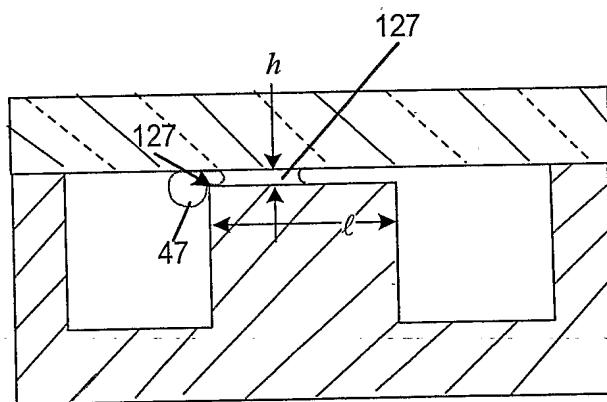


Fig. 4

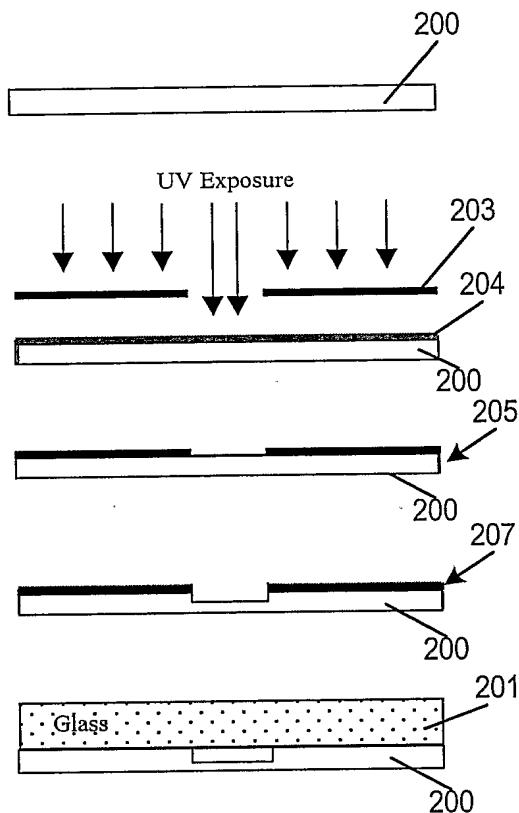


Fig. 5

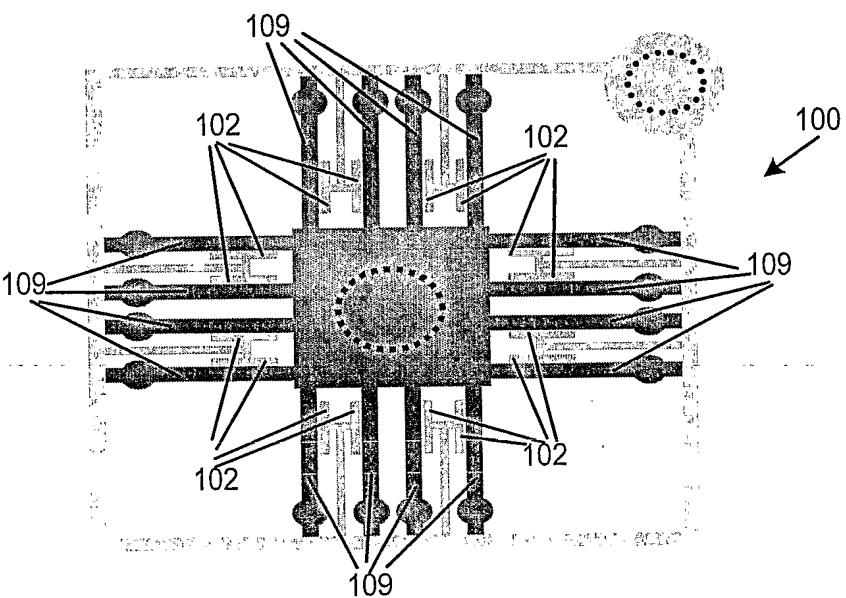
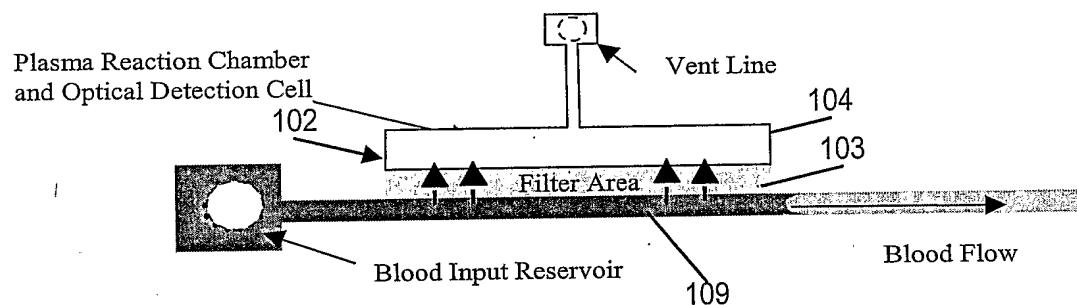
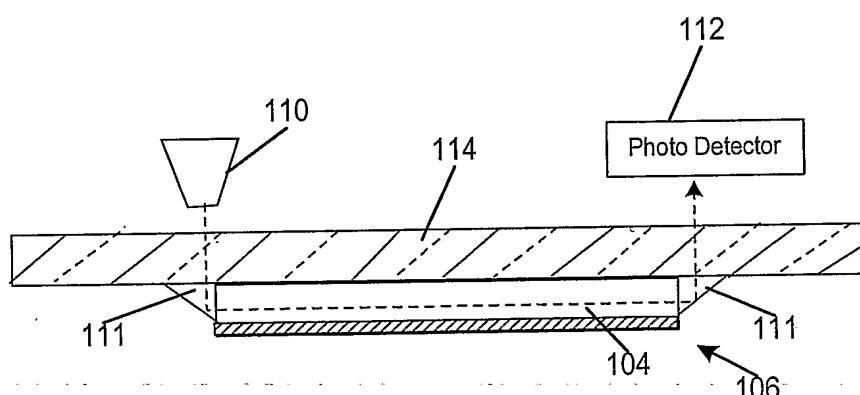
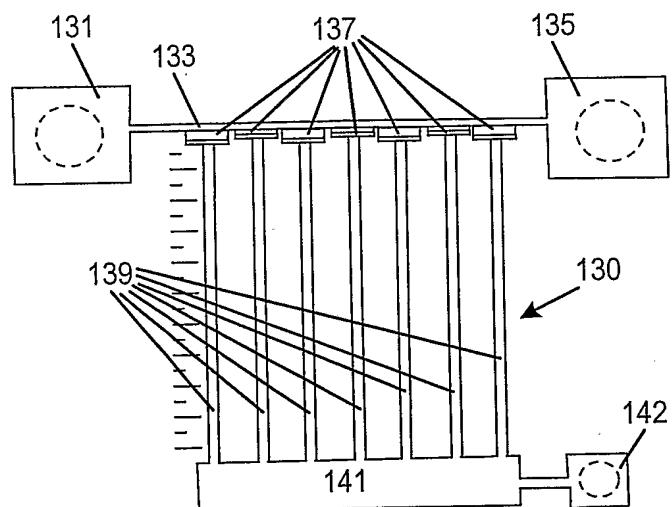
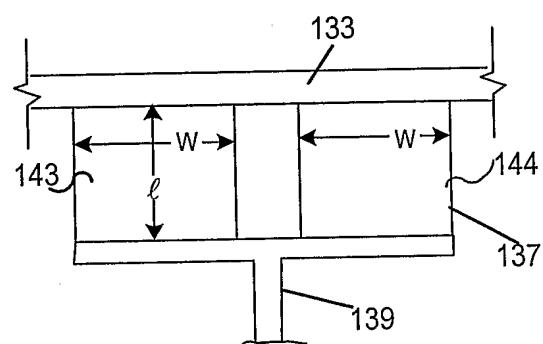


Fig. 6a

Device Detail**Fig. 6b****Fig. 6c**

**Fig. 7****Fig. 8**

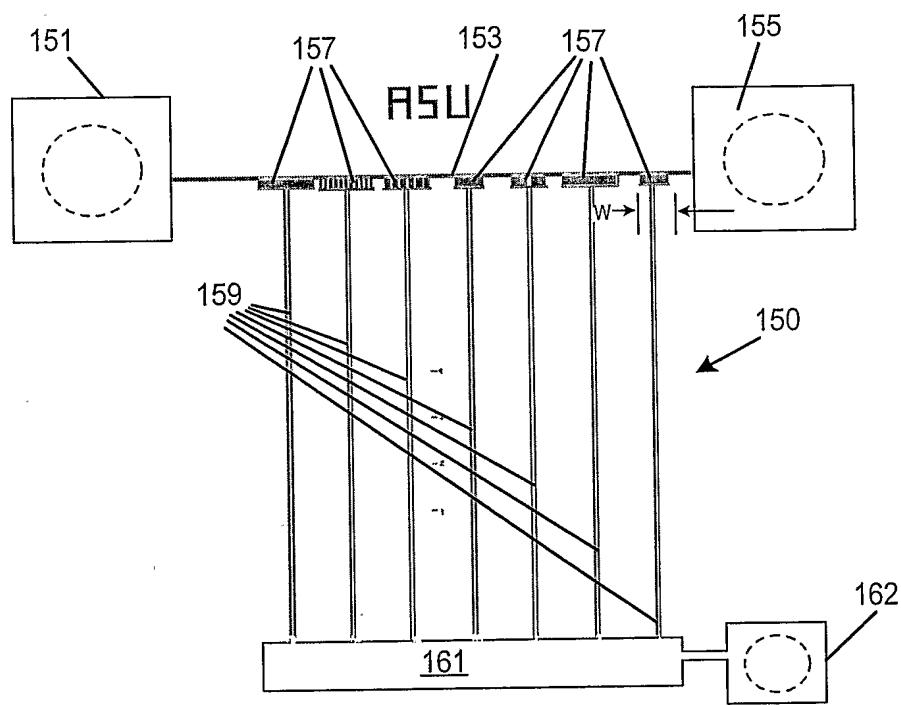


Fig. 9

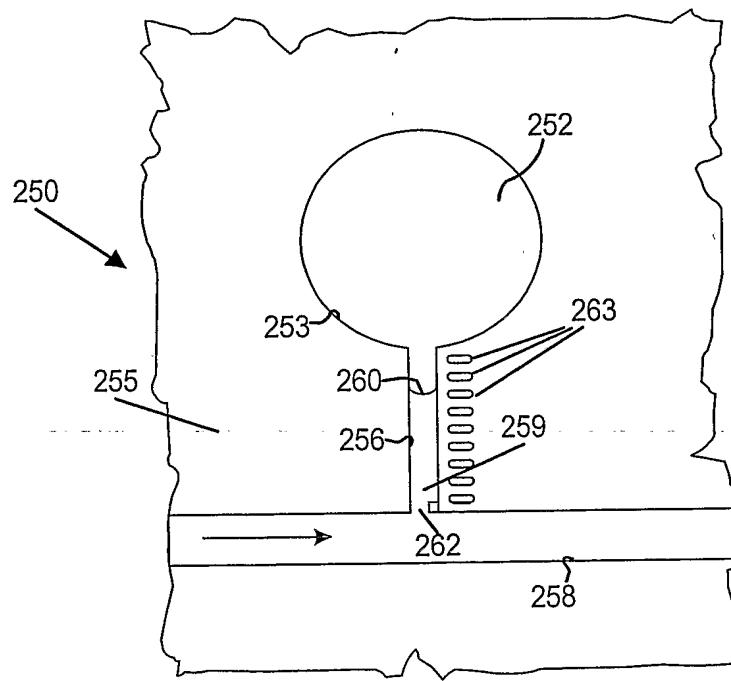


Fig. 10

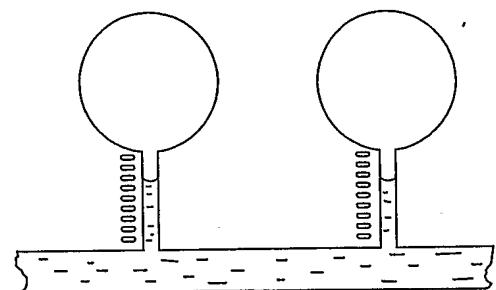


Fig. 11

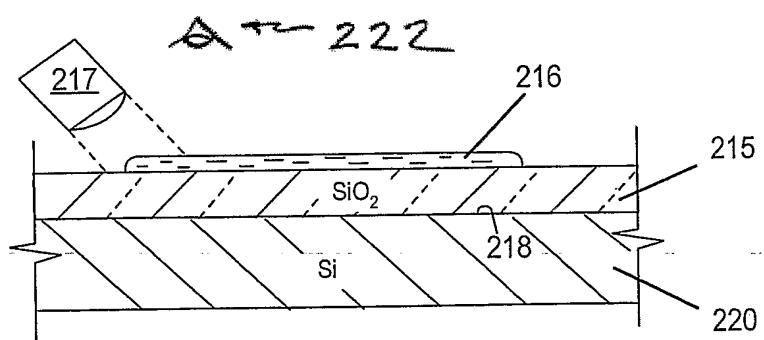


Fig. 12

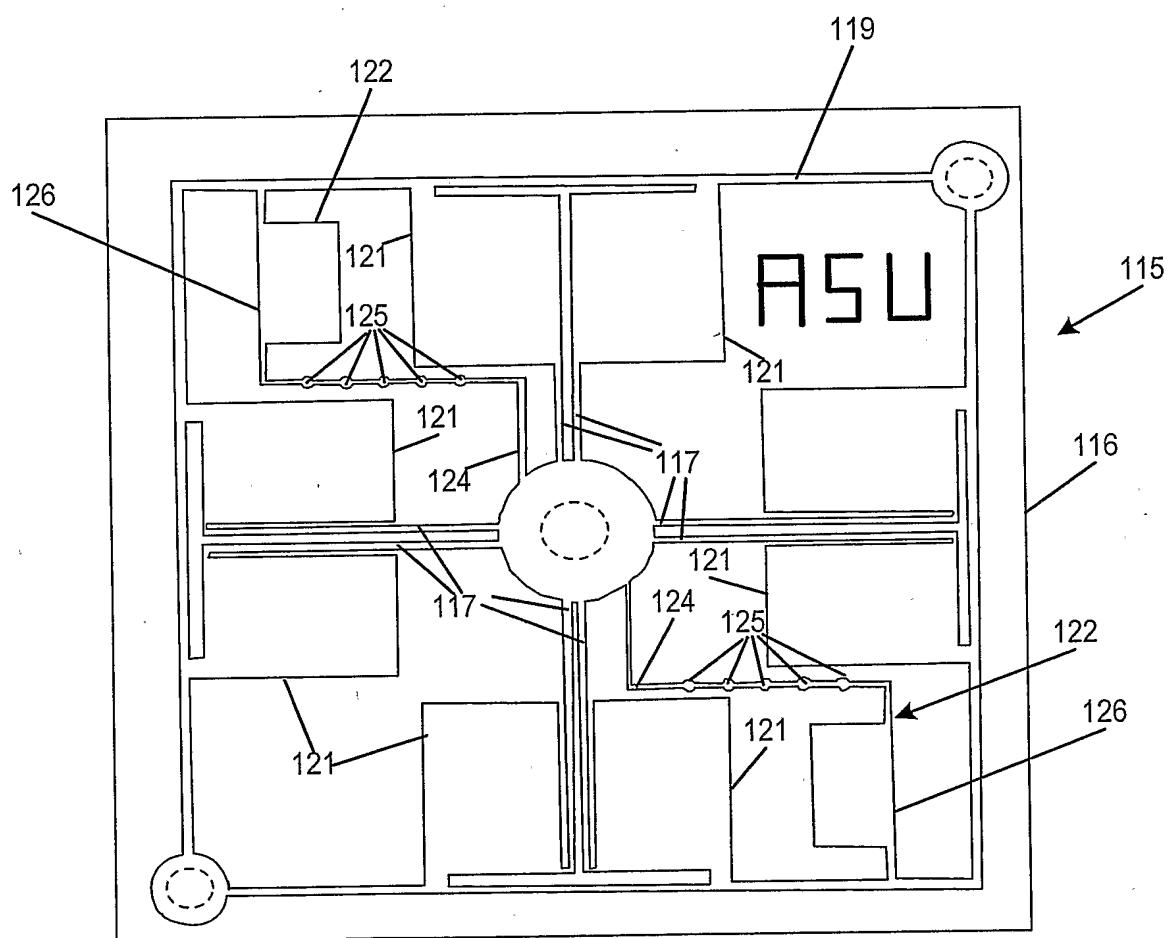


Fig.13a

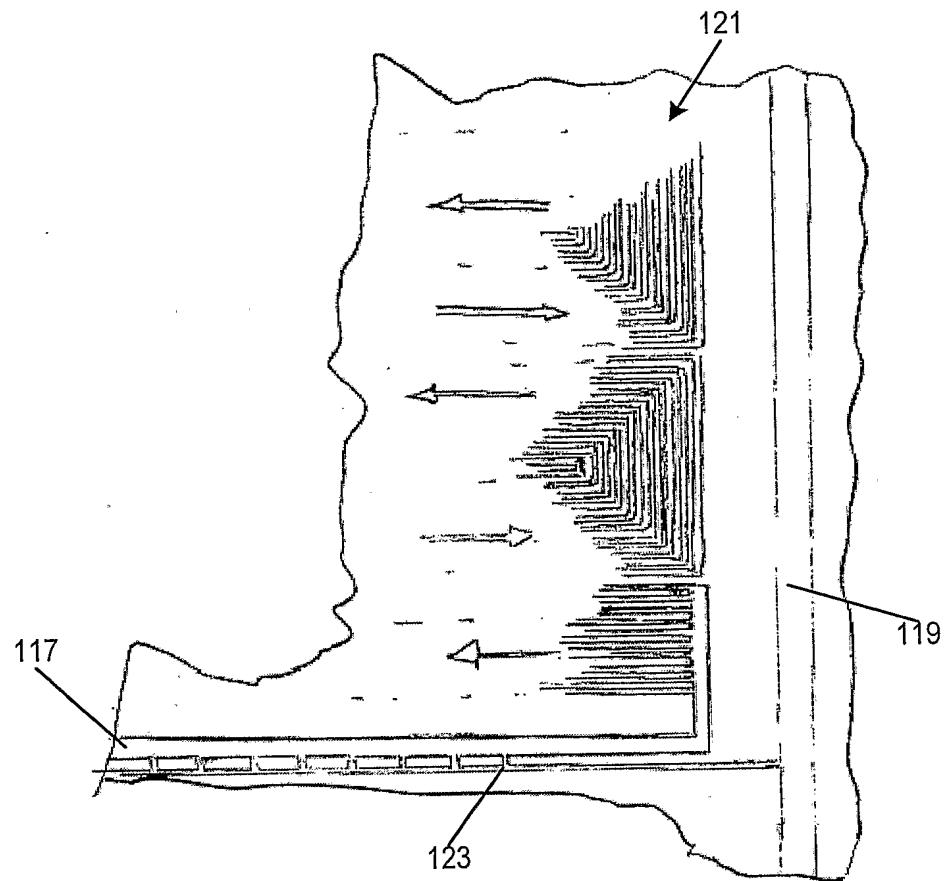


Fig. 13b

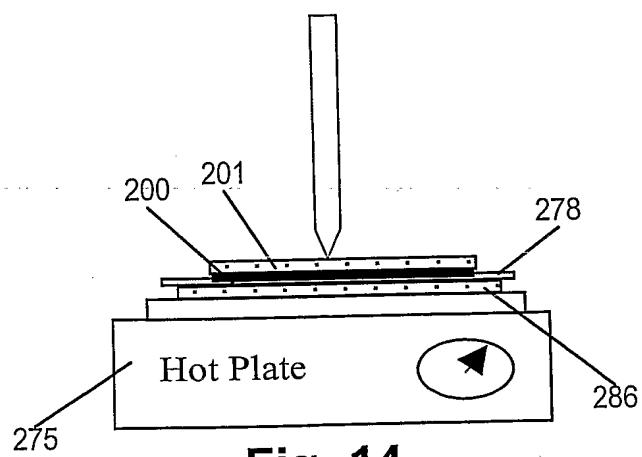


Fig. 14

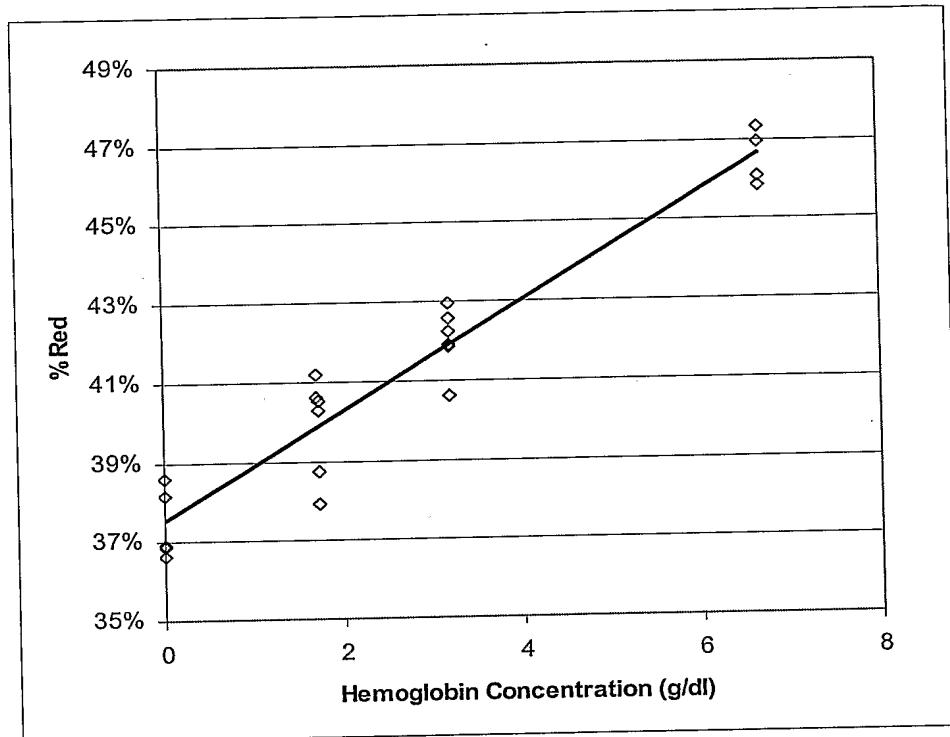
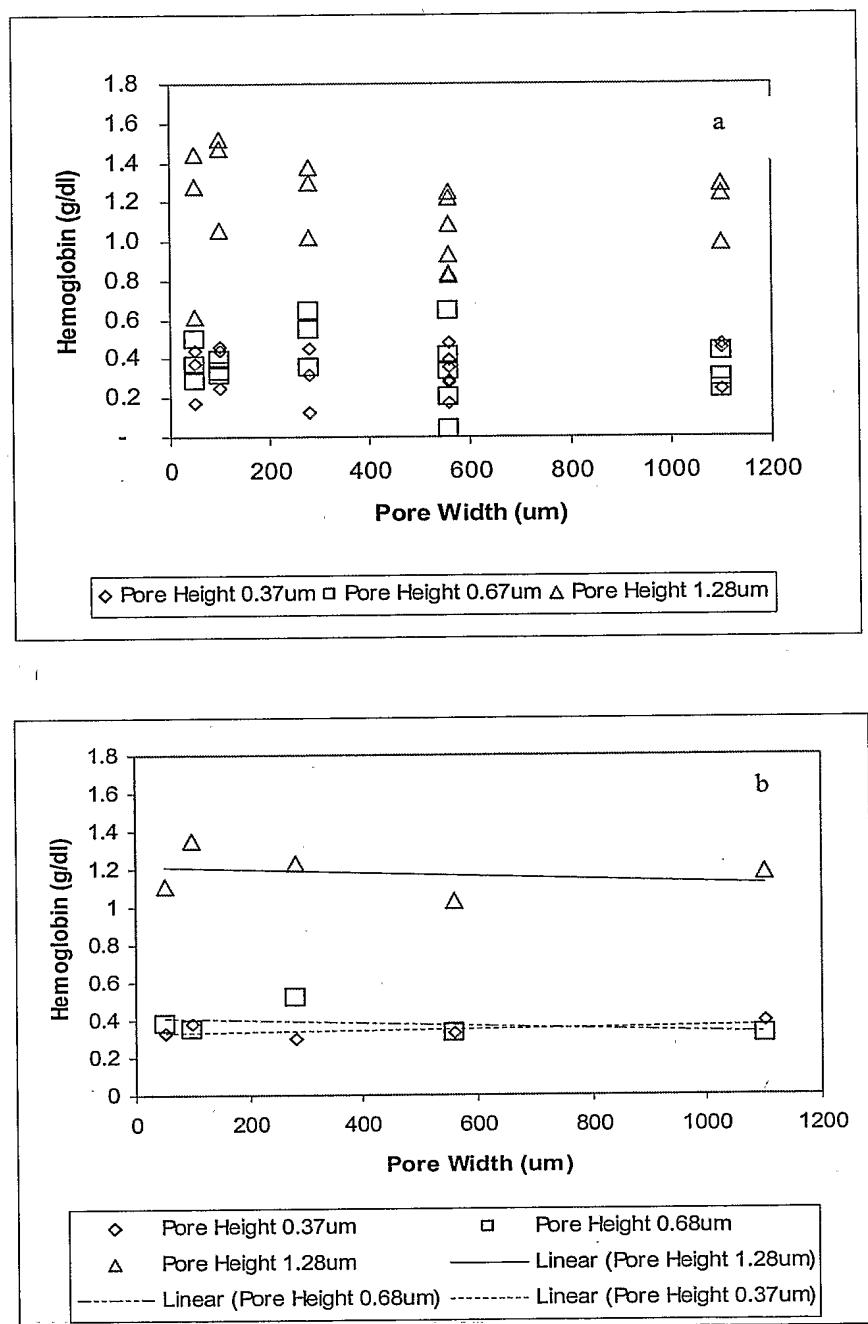
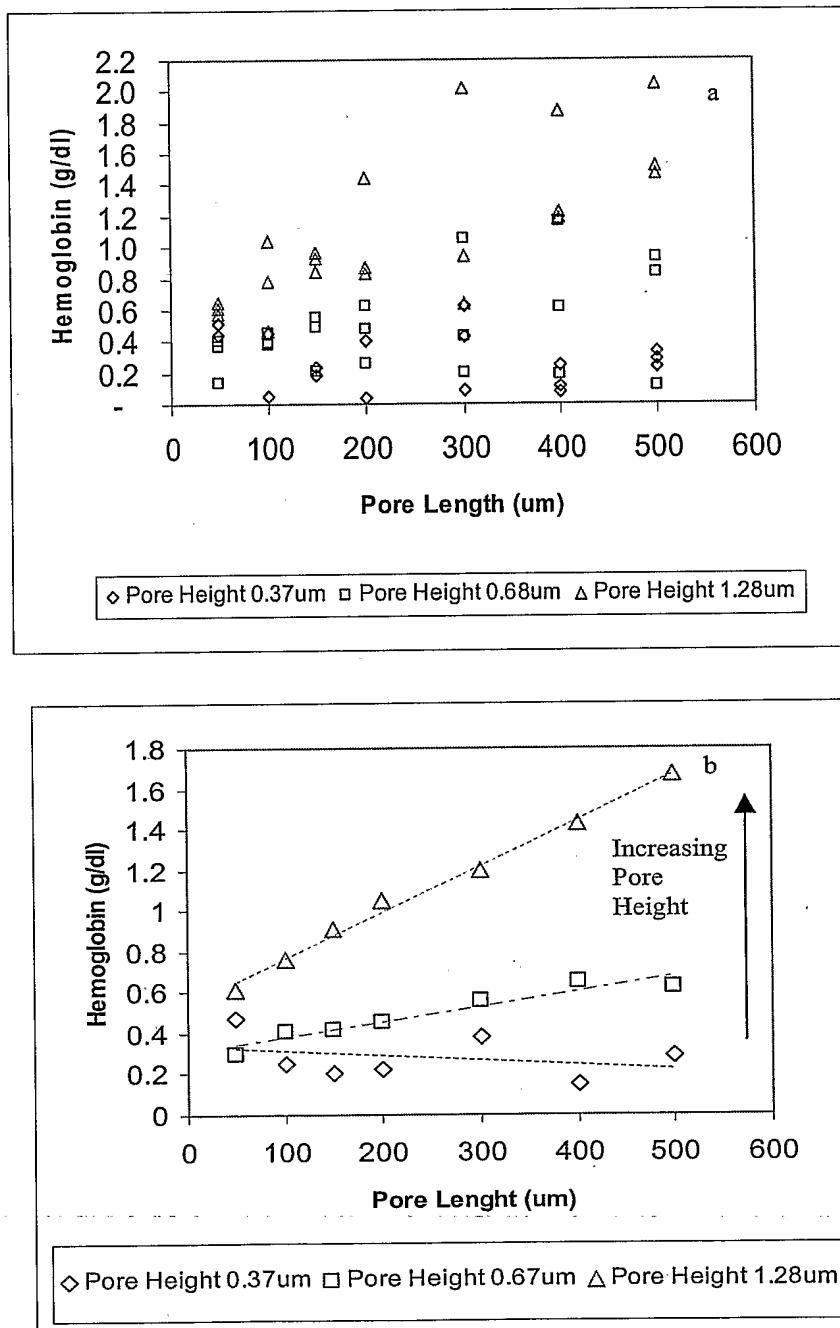
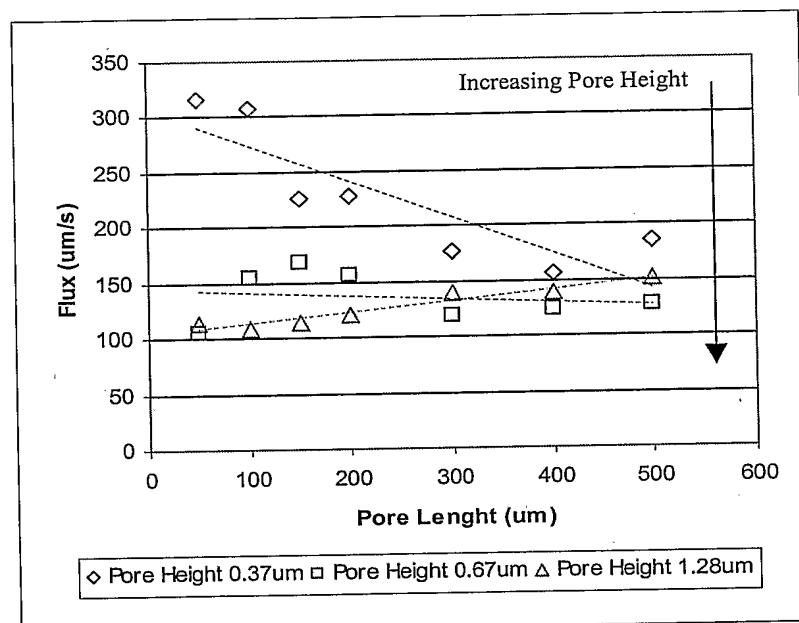
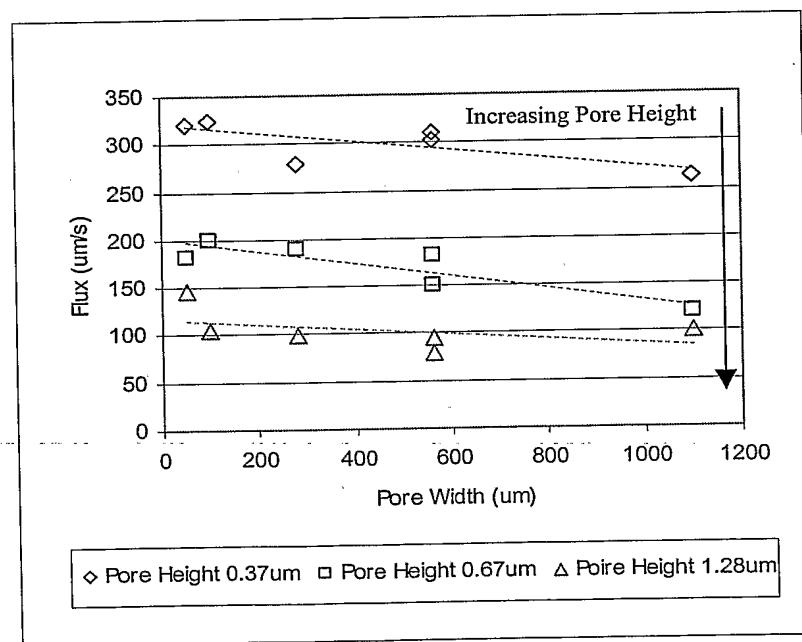
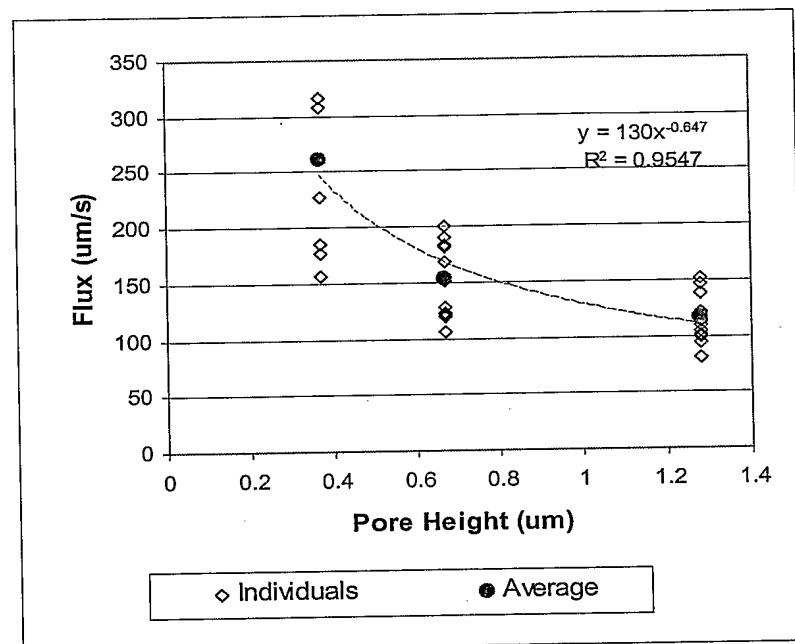
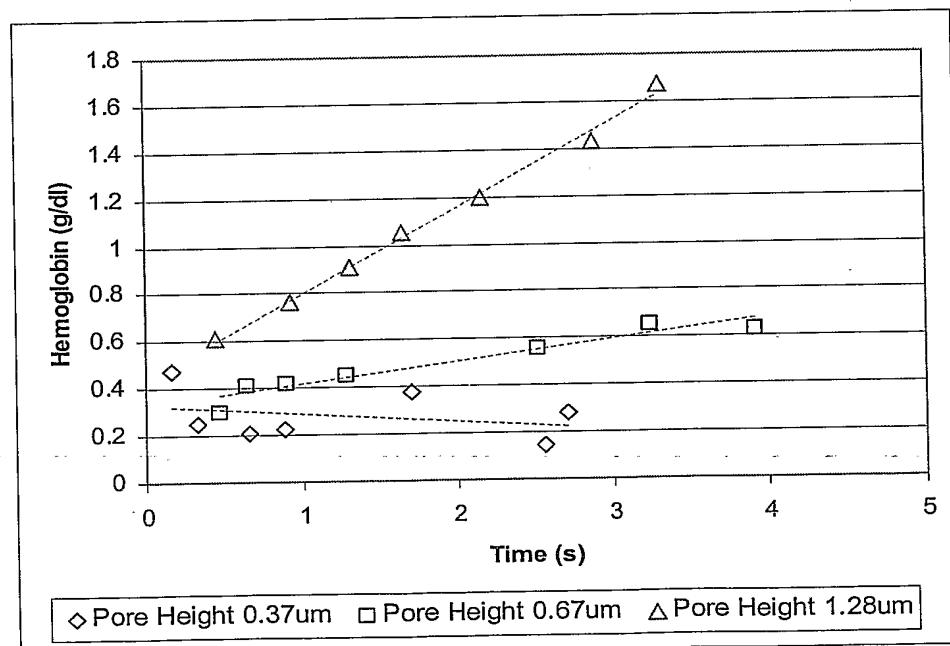


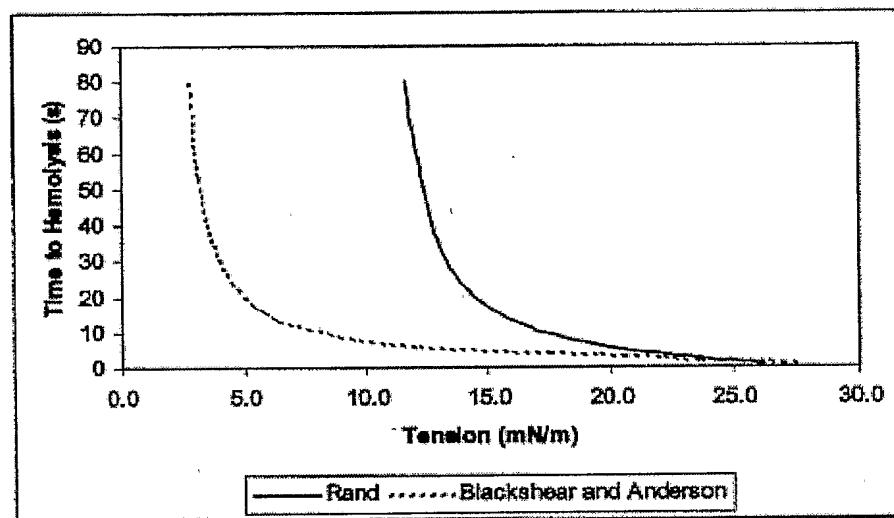
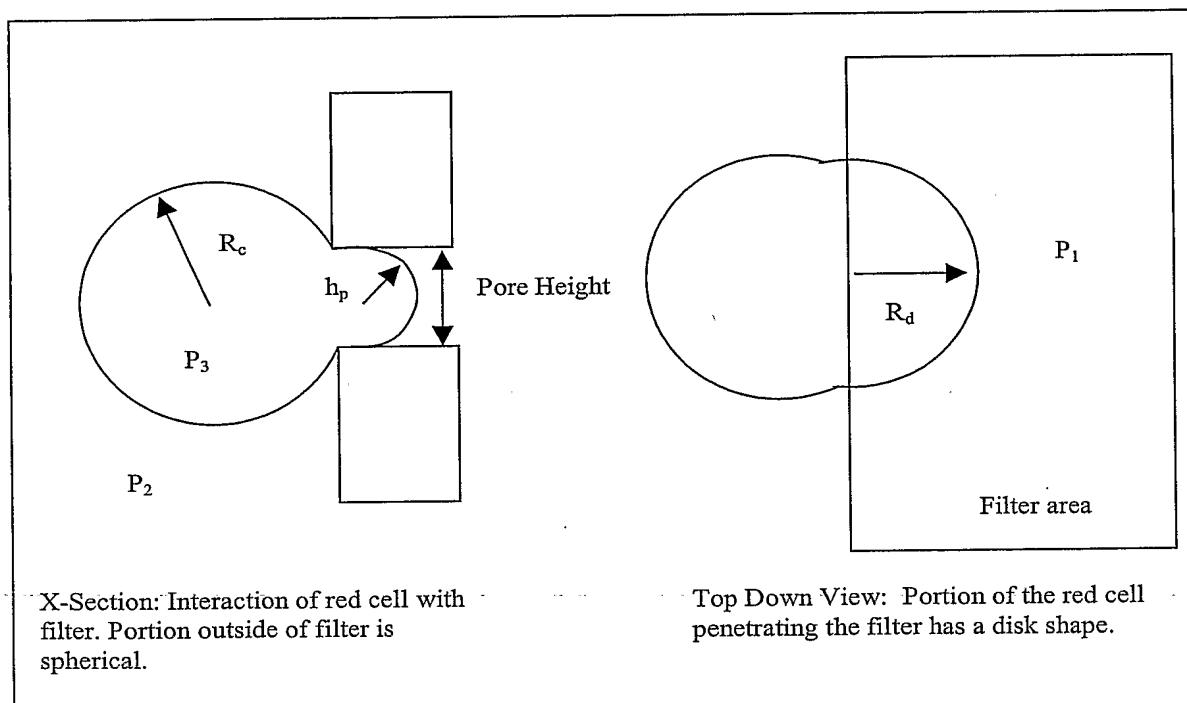
Fig. 15

**Fig. 16**

**Fig. 17**

**Fig. 18****Fig. 19**

**Fig. 20****Fig. 21**

**Fig. 22****Fig. 23**

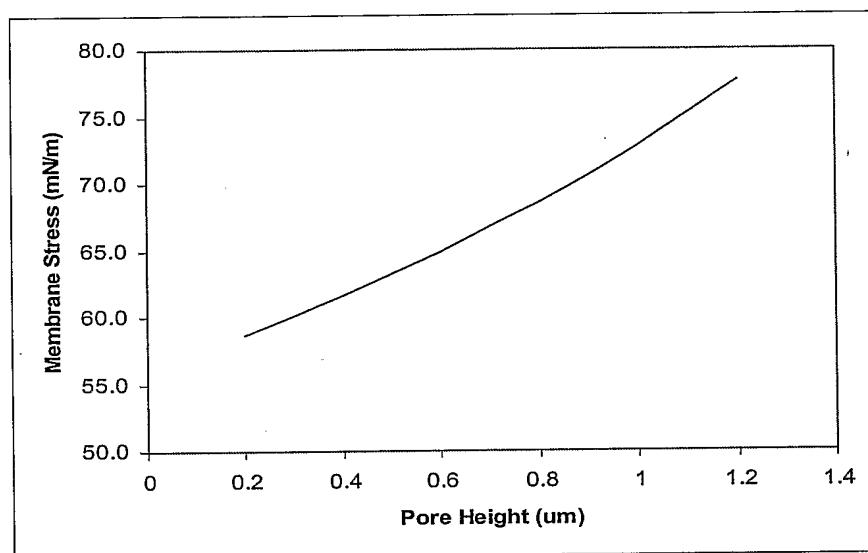


Fig. 24

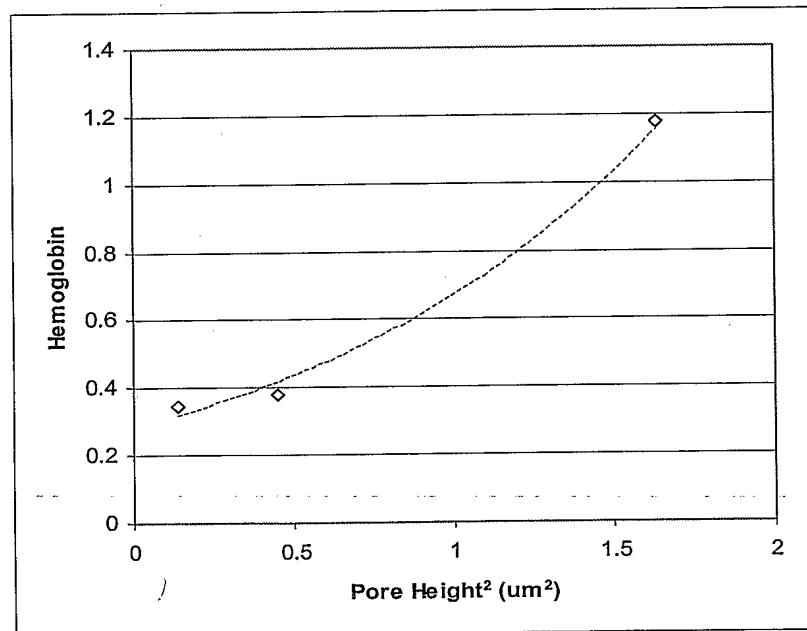


Fig. 25

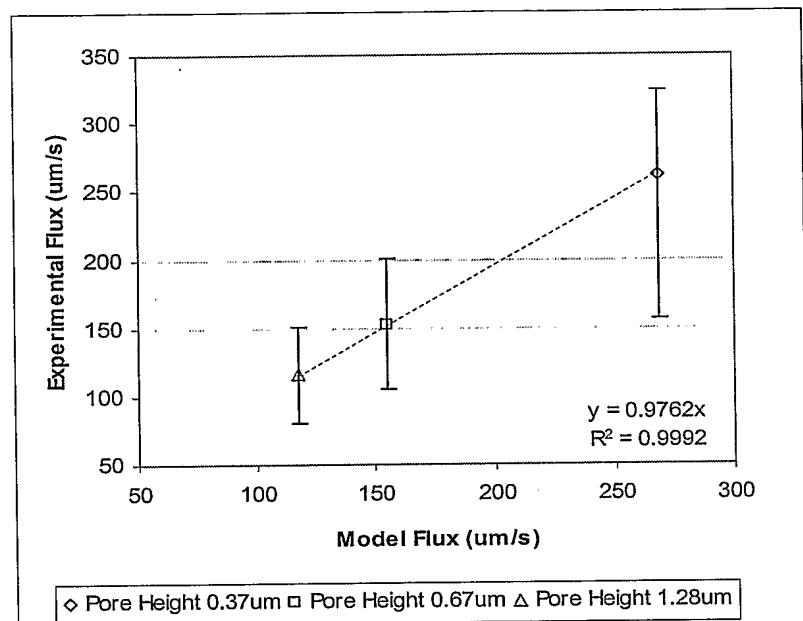


Fig. 26

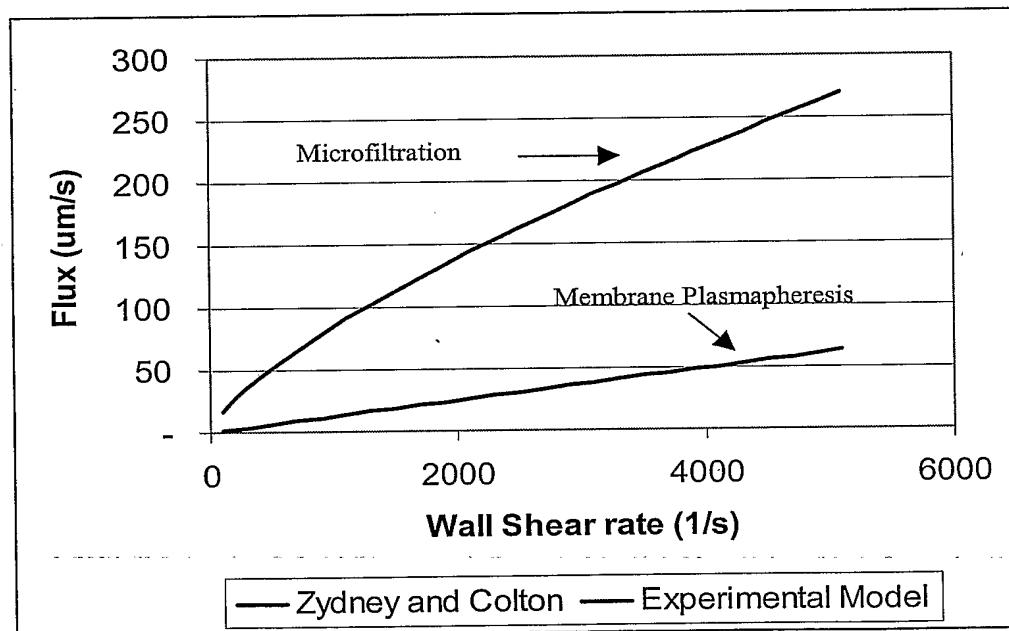
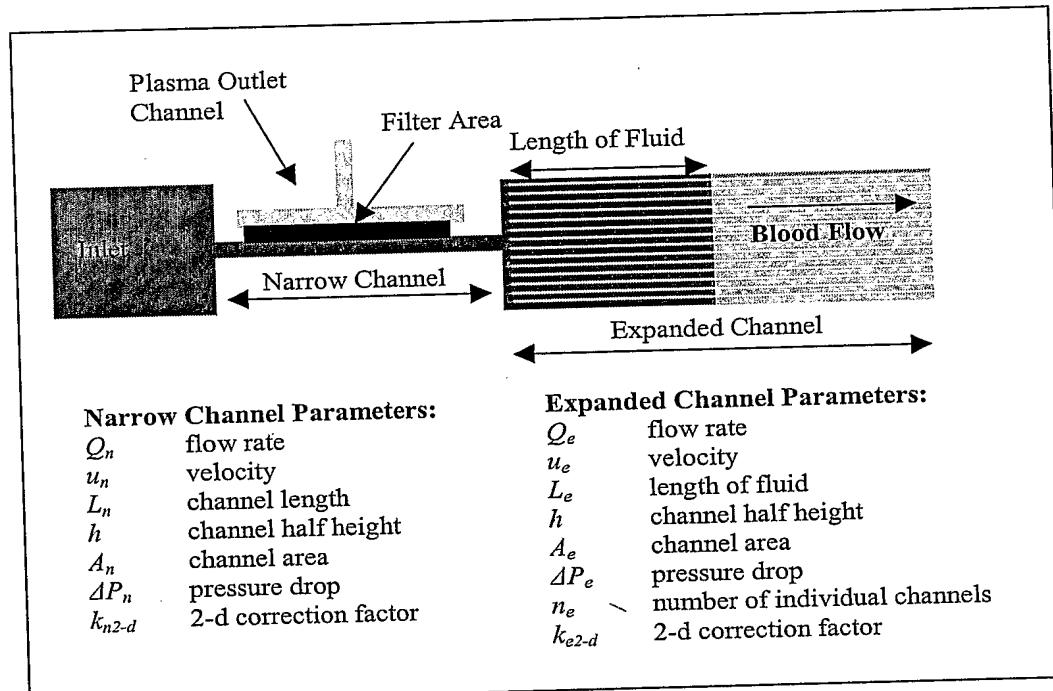
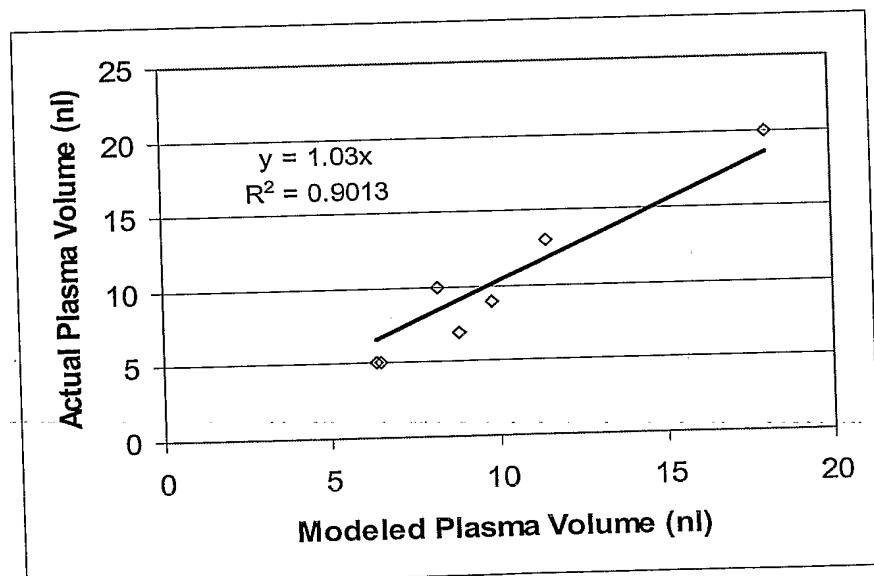


Fig. 27

**Fig. 28****Fig. 29**

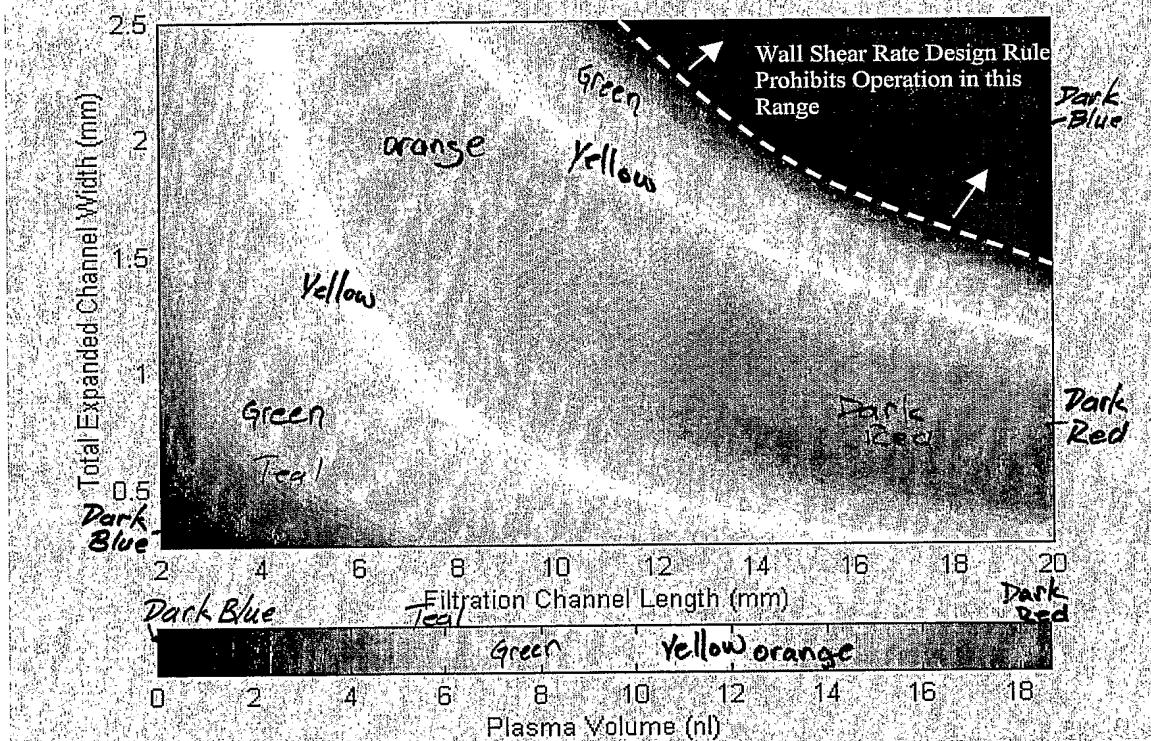
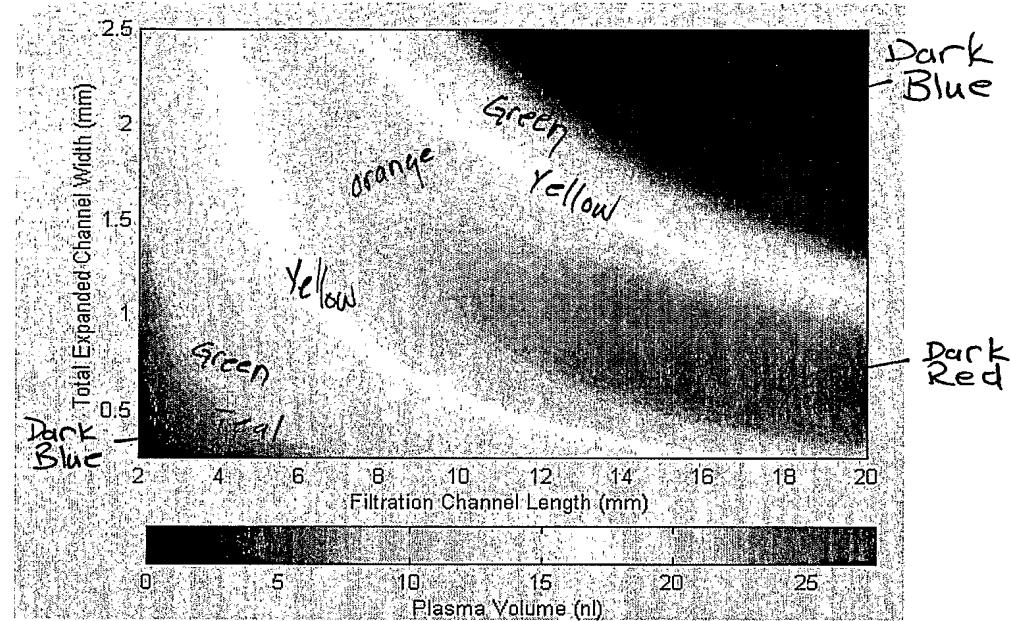
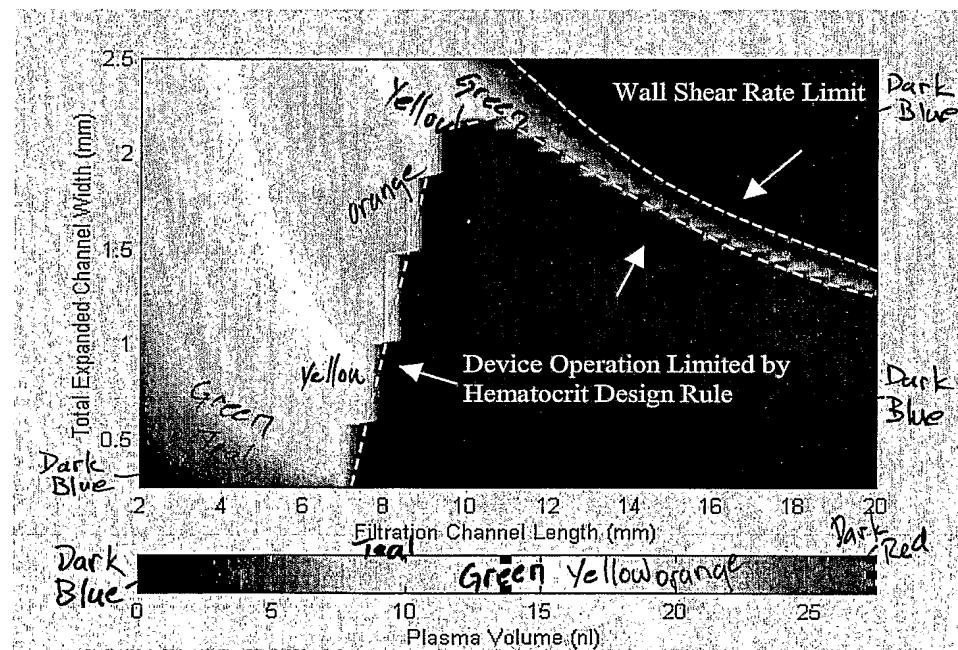


Fig. 30

**Fig. 31a****Fig. 31b**